Studies on Antifungal and Biochemical parameters of Plant material with special reference to Dermatophytosis

THESIS

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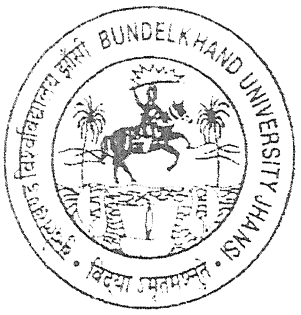
in Botany



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Certificate

I hereby certify that this thesis entitled "Studies on Antifungal and Biochemical Parameters of Plant Materials with Special Reference to Dermatophytoses."is an original piece of research work carried out by Sudha Chaturvedi under my guidance & supervision and also carried lab work for more than 200 days for the Degree of Doctor of Philosophy of Bundelkhand University, Jhansi (U.P.) She fulfills all the requirement laid under the clause Ph.D. Ordinance Bundelkhand University, Jhansi.

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CERTIFICATE

This is to certify that Sudha Chaturvedi ' Dixit ' working on " ***Study on antifungal and biochemical parameters of plants material with special reference to dermatophytoses*** *" for her Ph.D. Degree.She collected patients samples infested with dermatophytes from O.P.D. section of dermatology department M.L.B. medical college Jhansi under my supervision.From these samples she isolated pure culture of* ***Trichophyton mentagrophyte,Trichophyton rubrum,Microsporum gypseum and Microsporum nanum*** *in her lab.*

I wish her good luck for isolation of active plants material to inhibit these organisms so that they could be used in future for curing such infections.

Dr. Dinesh C. Govil
Professor & Head

डा० दिनेश चन्द्र गोविल
एम० डी०, डी० ...
प्रोफेसर व विभागाध्यक्ष
चर्म रोग ... रोग विभाग,
म० ल० बा० मे० कॉलेज, झाँसी

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Sudha Chaturvedi

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SECTION 1

INTRODUCTION

Introduction

Dermatophyte literally means, "Skin plants," which is a group of 30-40 closely related filamentous fungi, that can infect only superficial keratinised tissue the skin, hairs & nails. They cause a variety of clinical conditions collectively known as dermatophytosis. There are virtually no human populations completely free of this disease.

Emmons et. al., 1977, the superficial nature of dermatophytosis and the case with which fungi can be demonstrated in and on hairs enabled dermatologists to study and name the etiologic agent early in the era of microbiology. Charles Robin named the fungus now known as Trichophyton mentagrophyte in 1847 and Malmstem in 1845 gave the name Trichophyton tonsurans to another familiar dermatophyte in his book. These early studies described clearly several types of dermatophytosis and discussed the necessity for manual epilation of infected hairs and methods of topical therapy.

When Sabouraud began his systematic studies of dermatophytosis, he published after 1800 a series of paper correlating earlier studies and his own observations. Epilation of the scalp by X-ray was then possible and Sabouraud improved the method of treatment as well as method of studying the fungi in the laboratory. His work culminated in the publication in 1910 of his classic, His paper, although informative did not further clarify the taxonomy of the group of keratolytic fungi. Sabouraud

revolutionized the treatment of ringworm of the scalp in the thousand of infected children's in the school of Paris.

However the natural grouping of dermatophyte by Emmons in 1934 into three genera remain as the principle classification. Taxonomic review in 1968 by Azello listed 37 dermatophytes species including one species in the genus Epidermophyton, 15 in Microsporum and 21 in Trichophyton. Only 11 species of dermatophytes produce most of the dermatophytic infection in the world and less than eight of these are common in the united state.

Source of Infection :

The source of infection from dermatophytes includes contact with infected human being and contaminated fomites or soil. Those dermatophytes, which are associated principally with human beings and are rarely, if even isolated from animals or soil are said to be Anthropophilic. Zoophilic dermatophytes are seen normally in animal species, may initiate infections in human beings that are acute and strongly inflammatory. Rarely a keratinophilic soil organism may produce dermatophytoses in human beings and these are said to be geophilic because of their normally saprophytic existence.

The dermatophytes can initiate disease on skin; hair or nail. Depending upon species response Microsporum may infect the hair or skin. Epidermophyton floccosum may infect the skin and or and Trichophyton specie can infecting the skin or scalp nail as a small eczematoid lesion that spreads outward in roughly circular patterns with the viable fungal element at the

erythematous periphery Bocobo and Benham, 1949; Griffin, 1960; Rabell & Toplin 1970This ring shaped lesion was his to really thought to be caused by small insects or worms. So the common name for these infections became known as ring worm.

Clinical Types :

The clinical types & subtypes of dermatophytoses can be correlated only partially with specific dermatophytes. Species of dermatophytes are being mentioned only briefly and its clinical types are as under (Emmons 1977).

Tinea Pedis

(Athlete's foot, ring worm of the foot) lesions of this most frequent type of dermatophytosis often begin in the web between the fourth and fifth toes as tissues bordered by narrow zones of pealing epidermis.

Lesions of the above types may be present on the hands (Tinea manuum) Eczematoid lesions on the hands often are due to hypersensitivity of the patient to some allergens.

The usual fungi in tinea pedis & tinea manuum are Trichophyton rubrum, Trichophyton & Epidermophyton floccosum

Tinea unguium-

(Ringworm of the nails or Onchomychosis) Onychomycosis is often associated with mycotic hyperkeritonic peeling or inconspicuous branny desquamation of the hand & fingers. Infected nails may have a chalky crumbling consistency and

striated surface Hypertrophy of the nail bed resulting in a raised thicked nail which overlies a spongy mass of keratinized cells & debris. The species usually responsible for Onychomycosis are Trichophyton rubrum and Trichophyton mentagrophyte.

Tinea Corporis:

(Tinea circinata, ringworm of the glabrous skin) Typical Tinea circinata of the glabrous skin is characterized by a circular lesion characterizes typical Tinea circinata of the glabrons skin. Which exhibits varying degrees of inflammation, which may maintain its circular pattern of radial growth at the periphery of the lesion. The periphery lesions are located on the face, shoulder arms or other exposed parts of the body. The species usually responsible for Tinea corporis are Trichophyton mentagrophyte, Trichophyton rubrum and Microsporum canis.

Tinea imbricata:

(Takelan) In tropical areas of the Eastern hemisphere. Tinea imbricata is a common infection. It is characterized by lesions which are originally circinate but which become irregular or serpiginous and coalescent and don't heal at the centre. The patient does not develop local immunity and the lesions may cover large areas of the face, arms & legs for many years without remissions.

The name of the fungus is Trichophyton concentricum.

Tinea barbae:

(Sycosis, ringworm of the beard, barber's itch) Tinea barbae is found on the beard and other areas of the face & neck, which the patient acquires, from animals.

Tinea barbe is seen often in farmers and may be caused by Trichophyton verrucosum acquired from cattle or by Trichophyton mentagrophyte. Acquired from the horse or dog. Microsporum canis is an occasional cause of Tinea barbae.

Tinea cruris:

(Eczema marginatum, jockey itch, dhobie itch, ringworm of the groin) Tinea cruris is an acute or chronic infection usually pruritic dermatophytosis of the groin, perineal and perianal areas. When the above lesions are caused by Epidermophyton floccosum. They rarely extend beyond the areas mentioned above. But when the etiologic agent is Trichophyton rubrum lesions may extend widely over the body. The lesions usually are sharply marginated, epidermal scales are thin, real and if not secondarily infected generally dry up.

Favus:

(Tinea favosa) Favus is a severe type of chronic ringworm, which may be acquired during infancy and if not treated is carried throughout life.

In geographic areas where forms has been endemic for many generations many patient have a milder form of the disease hardly distinguishable from a seborrheic form of Tinea capitis.

Although Trichophyton schoenleini, Trichophyton violaceum & Microsporum gypseum cause favus the differences in severity are not clearly related to the species of the fungus favus of the nails are similar in its clinical appearance to Onychomycosis caused by other dermatophytes.

Favus commonly occurs in countries adjacent to the Mediterranean, South Eastern Europe, Southern Asia, Northern Africa and the orient.

Tinea capitis:

Those dermatophytes invading actively growing hair usually begin by growing in the stratum corneum of the scalp and entering the follicle to grow down the hair shaft to a point just above the keratogenous zone. That is a "living" portion of the hair shaft just above the hair bulb, which produce fibrils of keratin on the hair shaft and become established above the keratogenous zone. Some dermatophytes such as Trichophyton tonsurans and Trichophyton violaceum remain essentially inside the hair shaft while producing chains of arthrospores. This is called as endothrix type of invasion. The hair often become so fragile that they break off close to the scalp surface leaving black stubs with a chicken skin' appearance.

This condition is known as 'blackdot' ringworm and is typical of Trichophyton tonsuran and Trichophyton violaceum. Other dermatophytes Microsporum audouini, Microsporum canis, Trichophyton mentagrophyte and Trichophyton verrucosum secondarily break out into the surface of the hair and fragment

into spores (ectothrix). In the case of Microsporum canis and Microsporum audouini these spores are small (2 to 3 mm) and are packed-tightly into a mosaic and this small-spored ectothrix invasion is typical of these species. The spores of Trichophyton species producing ectothrix infections are larger (3 to 10 mm.) with a more apparent chain formation along the external hair shaft and include Trichophyton mentagrophytes and Trichophyton verrucosum.

Trichophyton schoenlinii produce distinctive patterns of hair invasion called favus. These hairs contain branched mycelium and empty spaces. Where the mycelium has degenerated. Such spaces readily fill with fluid when examined by KOH and bubbles can be seen in the fluid filled channels. These hairs are not as fragile as those in 'black dot' ringworm and the relatively long (2 to 8 inches) hairs take on a dull gray appearance.

Dermatophytes have been routinely placed in the class Deuteromycetes or 'imperfect fungi' because they were not known to reproduce sexually. More recently, however, an 'ascigerous' or "perfect form" has been discovered for several dermatophyte species Ajello. 1968; Dawson and Gentles. 1959; Griffin. 1960; Stockdale. 1961 When certain strains are grown on sterile hair (human or horse) or sterile moist soil, they may produce cleistothecia that contain asci with ascospores. The cleistothecia may reach 700μ in diameter and the asci contain eight ascospores of varying sizes and shapes, according to the species. The perfect form of the dermatophyte is given a genus and species name different from that of the imperfect form. The

generic term nannizea is given to the perfect form of Microsporum in honour of Nannize. Who described cleistothecia in a plate of Microsporum gypseum in 1957. Other authors more recently verified these findings (Griffin, 1960; stock dale, 1961) Additionally perfect forms have been found for members of the genus Trichophyton and these have the generic term Arthroderma. Some of the ascigerous genera are listed with their imperfect equivalents. The ascigerous states are not likely to be seen in the routine clinical laboratory because :

i) The dermatophytes producing an ascigerous state are rarely isolated in human infections.

ii) The demonstration of the ascigerous state required specialized techniques not common to the clinical laboratory. The knowledge of ascigerous states however is useful in identifying certain fungi otherwise difficult the identity.

This is accomplished by attempting to mate an unknown strain with a known strain. If sexual structure (Cleistothecia) is produced along the line where the colonies meet on the agar surface, the two organisms is considered to be of the same strain.

Emmons in 1934 defined in mycological terms three old generic names and proposed a natural simplified classification. Critical studies by Georg (1960); Ajello et. al., 1968 led to further clarification of specific nomenclature. Neal & Emmons 1939 isolated dermatophyte from 93 of 35h employees in an industrial plant.

Evidence that the dermatophytes are related to the Gymnoascaceae has been accumulating since 1899. Matruchat and Dassonville, et. al., 1899 Nannizzia et. al., 1927 observed cleistothecia in Microsporum gypesum and he named this ascomycetons form Gymnoascus gypseus. Griffin 1960 rediscovered G.gypspus and Szathmary and Herpay. 1960, observed this fungus and the ascomycetons state of Microsporum fulvum, a species generally considered identical with Microsporum gypseum. Stockdale et. al., 1961 using the hair bait' method. Stockdale. et al., 1963 published a second report on the Microsporum gypseum complex, in which she concluded that two species were included under the name Microsporum gypseum. In addition to N. incurvata stockdale, 1961, proposed the new combination, N. gypsea (Nannizzia).

Ajello and Cheng 1967 described Arthroderma ubenhiamia as the perfect state of Trichophyton mentagrophyte. Takashio et. al., 1972 investigated the mating reactions between various strains of Trichophyton mentagrophyte and two tester strains A and a types of A. benhamiae. It was found that Trichophyton mentagrophytes is a complex group producing sexual states which can be assigned to more than one species as is the case in Microsporum gypseum complex. Takashio 1973 later found the second ascomycetous species Arthroderma vanbreuseghemii. In the Trichophyton mentagrophyte complex. Hasegawa and Usui (1974) reported the perfect state of Microsporum canis to be a new heterothallic species of Nannizzia and the species were described as Nannizzia otal

Spontaneous or induced variants involving an altered growth rate, pigmentation, or conidial or colonial morphology were reported long before the discovery of sexual reproduction in dermatophytes (Emmons & Hollaender, 1939). It was later known that these changes are due to the mutation of nuclear gene using nutritional mutants obtained by irradiation.

Microsporum gruby, 1843 is characterized by fusiform or spindle shaped macroconidia which have usually thick walls Except in rare strains, the outer surface of the wall is pitted, a sperulate, or spiny at least near the distal end. Depending upon the species, the macroconidia are 7 to 20 x 35 to 125μ (rarely up to 160μ long) and they have usually 4 to 15 septa.

Microsporum gypseum (Emmons et. al., 1977)

(Bodin) Guiart and Griorakis, 1928. Synonymy: Achorion gypseum Bodin 1907; Microsporum flavescens Horta, 1911; Microsporum scorteum Priestley, 1914; Microsporum xanthoides Fisher. 1918.

Microsporum gypseum is normally a soil (geophilic) fungus and human being acquire this infection from soil contact (Farmers etc.) Presumably man and animal are infected from the soil, which serve as a saprobic reservoir of the fungus. Microsporum gypseum grows rapidly. Although it is not common Sinski - 84, 85. These fungi rarely invade the dermis Rinaldi 2000.

The colony is fawn brown buff or reddish brown and the pigment is conspicuously visible on the reverse side of the colony. The

surface is floccose, becoming powdery with production of many large septate macroconidia. Colonies are zonate with a powdery center and more wooly or floccose growth in the younger mycelium in peripheral zones. The colony rapidly becomes pleomorphic when hair is invaded it is ectothrix but sparse and there is no fluorescence with a wood's light. Macroconidia are produced in great number. They are 25μ to 60μ x 7.5μ to 16μ in size, broadly spindly shaped (although not so pointed at the distal end as those of Microsporum canis) with moderately thick walls and 4-6 septa. Micro conidia 2.5μ to 2μ x 4μ to 6μ are produced sparsely but sometimes more freely in subcultures after several transfers on media in the laboratory.

It is heterothallic, and mating by two strains produces cleistothecia. Nannizzea gypsea (Nannizzi) Stockdale 1963 is an ascomycetous state of Microsporum gypseum, which develops when compatible strains are mated on a suitable substratum such as soil mixed with hair or feathers. The cleistothecia are globose, pale buff, 300 to 750μ (rarely 900μ) in diameter. The peridial hyphae verticillately branched and branches curve back over the cleistothecium.

Nannizzia incurvata stockdale 1961 is another ascomycetous state of the species complex now recognized as Microsporum gypseum. It develops when compatible strains are mated on a substratum of soil mixed with hair or feather. The cleistothecia are globose, pale buff 350 to 650μ in diameter. The peridial hyphae verticillately branched. Branches curving towards the main axis and away from the cleistothecium.

Microsporum nannum

Microsporum nannum was isolated from a lesion of the scalp of a boy and from the body of an adult in Cuba, later from swine in Kenya and subsequently from man and swine in other parts of the world. Microsporum nanum is a zoophilic fungus and human being acquired this infection from animal contact through generally pigs. It produces Tinea corporis in human beings.

The genus Microsporum is immediately recognized by the presence of large (8μ x 8μ to 15μ x 15 to 150μ spindles shaped, rough walled macroconidia with thick (up to 4μm) walls that contain 4 to 15 septa. The exception is Microsporum nanum, which characteristically produces macro conidia having two cells. It's macro conidia are ovate or elliptical 12μ to 18μ x 5μ to 7μ with 1 to 2 cells (rarely 24μ long and with 4 cells), and with outer walls which are verrucose (rarely smooth) The micro conidia when present are clavate 2μ x 5μ and are borne on the hyphae either laterally or on short conidiophores. Species of Microsporum develop either slowly or rapidly and produce aerial hyphae that is velvety, powdery, glabrous or cottony varying in colour from whitish, buff to a cinnamon brown, with varying shades on the reverse side of colony.

Nannizzia obtusa 1961 is the ascomycetous state of Microsporum nanum. Its cleistothecia are globose pale buff and 250 to 450μ in diameter.

Trichopyton is characterized by clavate macroconidia, 4 to 8 x 8 to 50μ with smooth walls usually not exceeding 2μ in thickness and zero to four septa. The cells of macroconidia are multinucleate as in other dermatophytes. The microconidia are spherical 2.5 to 4μ in diameter or clavate 2 to 3 x 3 to 4μ Species of Trichophyton attack skin, hairs or nails.

Trichophyton mentagrophyte

Trichophyton mentagrophyte (Robin) Blanchard, 1896. Synonymy: Microsporum mentagrophytes Robin, 1853; Achorion quinkeanum Blanchard, 1896; Trichophyton felineum Blanchard 1896; Trichophyton gypseum Bodin, 1902; Trichophyton granulosum sab, 1909 Trichophyton radiolatum sab., 1910; Trichophyton lacticolor sab. 1910; Trichophyton niveum sab; 1910 Trichophyton radians sab., 1910; Trichophyton denticulatum Sab., 1910; Trichophyton asteroides Sab; 1910 Trichophyton farinulentum Sab; 1910; Trichophyton interdigitale priestly 1917; Trichophyton kaufmann- wolf ota, 1922; Trichophyton pedis ota 1922.

Trichophyton is the most common and complex, containing over 15 species and several varieties with in the species. Trichophyton mentagrophyte Rippon 1988 and Weitzman I. Summerball 1995. The species of Trichophytons are the most commonly isolated of all dermatophyte species from human ringworm infections. Their identity however, presents the most difficult of all the dermatophytes. Most of the species common to human infections fail to produce macroaleuriospores and there are limited

physiologic tests available to assist in the differentiation of these species. Trichophyton is characterized by clavate macroconidia with smooth walls usually not exceeding 24μ in thickness and with zero to four cells of macroconidia.

Trichophyton mentagrophytes is nearly as common as Trichophyton rubrum as an etiology agent of dermatophytosis in human beings and is the most common agent of Tinea pedis. While being an important cause of most other forms of tinea as well. The colonies of Trichophyton mentagrophytes vary from white floccose colonies with no distinctive microscopic features except a few clavate microconida of cream coloured, yellowish or peach-coloured granular, flat colonies which bear spores freely. The morphologic features of these strains include clavate 3 to 4 septate macroconidia 6μ to 8μ x 20μ to 50μ in size; spherical or clavate microconidia; spirally coiled hyphae and nodular organs which are abortive ascogonia. The colors may include tan and reddish brown. The reverse pigmentation is equally variable and confusing. The colours may range from colourless or white, through various shade of brown to a red pigmentation. Trichophyton mentagrophytes fail to produce a red pigment on corn meal dextrose agar. Whereas Trichophyton rubrum produce a red pigment consistently on it. Trichophyton mentagrophyton is further characterized by production of enzymes, which permit it to penetrate hair in-vitro by formation of deep narrow conical pits, and by production of urease. Most strains of Trichophyton rubrum either lack these enzymes or produce them slowly.

Macroconidia vary within the strain and from strain to strain from single celled spores 4μ to 8μ in size to 2μ to 5μ celled spores 8μ x 50μ in size. They may be most easily found in young cultures five to ten days old. Microconidia may be clavate and borne laterally on undifferentiated hyphae in floccose strains or nearly spherical on conidiophores in powdery or granular strains. The conidiophores may be once or twice branched to produce clusters of these sub spherical microconidia or microalerisopores. The short branches arising at almost right angles.

Trichophyton mentagrophyte falls within the "Small spored ectothrix" group, although most of the floccose strains of the type commonly isolated from Tinea pedis, do not spread to the scalp and do not invade hairs of the glabrous skin. The species includes strains, which do invade hair follicles and hairs and cause severe host reaction, which may be supportive expulsion of hairs and spontaneous termination.

Arthroderma benhamiae Ajello and cheng 1967, is the ascomycetous state of Trichophyton mentagrophytes. Cleistothecia are white spherical, 400 to 500μ in diameter. Arthroderma vanbreuseghemii (Takashio 1973) is another ascomycetous state of the Trichophyton mentgrophyte complex. The morphological characteristics are similar to those of A benhamiae except it has slightly larger ascospores measuring 2 x 3.5μ in diameter. Interspecific crossing does not occur between A. benhamiae and A. vanbreuseghemii.

Trichophyton rubrum

Trichophyton rubrum (castellani) Sab; 1911. Synonymy: Trichophyton rubrum cast; 1910; Epidermophyton pernete cast; 1910; Trichophyton purpureum Bang, 1910; Trichophyton rubidum Priestley, 1917; Trichophyton plurizoniforme Mac carthy, 1925; Trichophyton anoroseum Mac carthy, 1925;Trichophyton coccineum Katon; 1925; Trichophyton spadix Katoh, 1925; Trichophyton multicolor magathaes and Neves, 1927; Trichophyton Kagawaense Fujii, 1931.

The anthropophilic dermatophyte Trichophyton rubrum is considered to be the most common cause of dermatophytosis in the United State and is normally isolated from Tinea corporis and Tinea pedis.

Trichophyton rubrum can be highly variable in morphology, but is normally seen on sabouraud's Agar medium as a slow glowing, heaped white to reddish floccose or velvety colony. The cherry red pigment is most apparent on the reverse side of the colony. The pigment usually develops after some weeks of growth but may be developed at all for some strains of Trichophyton rubrum particularly if the patient is on griseofulvin therapy. This red pigmentation can be more consistent at Trichophyton rubrum is grown on corn meal dextrose agar, while Trichophyton mentagrophyte fails to produce red pigment on this medium (Bocobo and Benham, 1949). On agar slant may appear first at the margin of a colony at the dry tip of the slant or at the center and in a concentric circle on the reverse side of a colony.

Macroconidia are typically long and narrow (4μ to 6μ x 15μ to 30μ) are sparse or lacking except on enriched media such as heart infusion tryptose agar. Microconidia are clavate (2μ to 3μ x 3μ x 5μ), borne laterally on undifferentiated hyphae or on simple lateral conidiophores. They may be almost sessile or on short stalks. Ascogonia produced but are less numerous in most strains than in granular types of Trichophyton mentagrophyte.

Trichophyton rubrum rarely invades hairs, but when it does so, it is ectothrix in nature. It is frequent cause of Tinea pedis, onychomycosis, and may extend widely over the body, particularly in the perineal, groin and waist areas. It is a rare cause of subcutaneous and systemic of infection.

There are relatively few therapeutic agents developed for dermatopytosis treatments. Eukaryotic nature of the fungi is one of the reasons and it has been difficult to develop antifungal agent specific for fungal structures (Kobayaski and Medoff, 1977)

The first therapeutic agent, sensitive against mycoses was Potassium Iodide the use of which was chiefly confined to sporotrichosis (Conant et. al., 1971). However, during the last 50 years a large number of synthetic compounds Viz. Benzimidazole, diamidines, diothiocarbonates, hydroquinolines and pyrimidines have been reported to be effective against dermatophytes. Unfortunately, the use of such synthetic compounds in human chemotherapy remained limited (Stevens et. al., 1976 Botter 1980;Brass et. al., 1980; Creatsas et. al.,

1980; Fainstein and Body 1980; Carybill et. al., 1980; Heel and Brogden, 1980; Lawson and Body, 1980; Mucke 1980; Peterson et. al., 1980; Utz. 1980; Wojtulewaski et. al., 1980) Some antifungal, antibiotics Viz. Griseofulvin, Nystatin, and Amphotericine prevalent in the treatment of human mycosis have recently been found to possess various side effects Viz. Headache, gastro- intestinal upset and transientrashes (Roxburgh and Borrie 1973) Many polyens antibiotics produced by species of Strephomyces have been of potential clinical usefulness for the control of such diseases. However at present only two products Viz. Amphotericin B and Nystatin are being used as chemotherapeutic agents in human mycotic infections. Recently, Nystatin has been found to show some side effects (Pareck 1980) and Amphotericin B also exhibits the whole range of side effects including fever, headache, malasia, chill, sweating, depression, nausea, vomiting, anorexia and anemia (Emmons et. al., 1977) Treatment usually requires systemic antifungal therapy. Although a topical transfungal drug delivery system including 8% Ciclopirox and 5% Amorolfine nail lacquer has been developed for distal nail infection. Qadripur et. al.,1981 and Zaias, 1985. Both the systemic and the new topical treatments require several months of medication. Systemic antifungal agents may cause serious idiosyncratic effects such as hepatitis or drug induced lupus erytheromatous. (Zaias 1985)

Terbinafine is an allylamine effective against dermatophytes and some moulds. The drug is well absorbed after oral administration, binds strongly to plasma proteins and diffuses

readily into formed nail plate from both the nail bed and matrix. (Balfour and Faulds 1992) & (Gupta, et. al., 1997) Most side effects have been characterized as minor and transient, although some patients have been reported to develop Neutropenia, Pancytopenia and Hepatotoxic reactions while receiving the drug. Blood counts and liver function tests are thus necessary during treatment.

In the modern times Grieseofulvin is a first line well tolerated therapy for dermatophyte infections specially for Microsporum species. Which have many side effects such as headache, nausea and vomiting are most frequently countered. This systematic antifungal antibiotic Griesofulvin is by no means the perfect treatment of such infection (Roxburgh and Borrie 1973). Drug results in stunting and shrinking of fungal hyphae. It does not impede but enhances deoxyribonucleic acid synthesis. Occasionally patients don't response to these modern drugs and at the same time may causes harmful effects. So it is necessary to develop an effective and safe therapy. In view of this the present study is designed to organize a screening programme to test the antifungal principle present in plants.

Plants tested so far for their antifungal activity have not been worked out in detail. It is not known that which specific part is active whether they are safe or not and it which dilution of plant extract the growth of dermatophytes is inhibited which could be recommended for use. Some of the plants which have already been reported to posses anti-fungal agents against the

dermatophytes are :

Asteracantha longifolia against Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum Epidermophyton floccosum and Candida albicans (Venkitaraman and Radhakrishana, 1972).

Cassia abrus; Cassia auriculata and Cassia fistula against Microsporum tonsurans, Trichophyton rubrum and Trichophyton magninii (Lillykutty and Santhakumari, 1969) Cocos nucifera against Trichophyton mentagrophyte and Microsporum gypseum (Gaind and Single, 1968) Euphorbia thymifolia against Trichophyton mentagrophyte (Rao and Gupta, 1970).

Water extract of Cassia angustifolia was found effective against Trichophyton purpurceum (Itok and Najayo, et. al., 1951).

The root bark of Cassia fislula (Caesalpiniaceae) was found effective during in vitro studies. Its 100mg of acetone extract of root and stem bark showed antifungal action against Trichophyton tonsurans, Trichophyton rubrum and Trichophyton megnini and was more potent than 10 g. griseofulvin. (Narayanana and Seshadri. 1972).

Two flavonoid glycoside fractions isolated from the acetone extract of the root bark also possessed marked antifungal activity against Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum and Trichophyton tonsurans. Extract of roots and leaves of Leptadenia reticulata (Asclepiadaceae) was found active against ringworm (Patel and Dantwala:1958).

Clinical trials has been done with a preparation from the fresh leaves of Azaadiracta indica in common skin conditions eczema, ringworm infection by Singh, et. al., 1979).

Ocimum americanum oil showed antifungal activity against Trichophyton mentagrophyte, Trichophyton rubrum Epidermophyton floccosum and Candida albicans (Narasimha Rao & Subba Rao, 1972). In another study the essential oil was found to be active against Epidermophyton floccosum, Trichophyton mentagrophyte, and Microsporum canis (Singh et. al., 1983).

Eugenol acetate, geranyl acetate and methyl heptanone, the three components isolated from the essential oil of O, americanum were evaluated for their antimycotic activity against keratinophilic fungi, Among the three compounds strong potent fungitoxic activity was in eugenol and geranyl acetate (Jain et. al., & Agrawal 1978) against dermatophytes also. The activity of these compounds however, was some what less potent than that of griseofulvin (Jain et. al., 1980) Geranyl acetate was found to be highly toxic to Trichophyton rubrum.

The oil of O. basilicum showed antifungal activity against Microsporum gypseum (Sawhney et. al., 1977) Trichophyton mentagrophyte, Microsporum canis and Epidermophyton floccosum (Singh et. al., 1983).

The oil of O. gratissimum plant showed antifungal activity against Microsporum gypseum (Sawhney et. al., 1977) as well as against Epidermophyton floccosum. Microsporum canis and

Trichophyton mentagrophytes at a concentration of 1000 ppm (Singh et. al., 1983).

The essential oil of O. Kilimandschoricum showed potent antifungal activity against Epidermophyton rubrum, Microsporum gypseum. The oil was most effective against Microsporum gypseum (Suri & Thind; 1979).

The essential oil of Ocimum sanctum from the plant also showed antifungal activity against dermatophytes Viz. Epidermophyton floccosum. Trichophyton mentagrophyte & Microsporum canis (Singh et. al.. 1983).

In view of the above findings the present work has been planed in the following lines.

1- a) Collection of plants and preparation of powdered material.
   b) Preparation of water extracts from plants powders.
   c) Preparation of solvent extracts from plants part
   d) Preparation of hot solvent extract by soxhlet apparatus.
   e) Extraction of Essential oils from plants material
   f) Preparation of composite samples.

2- Isolation of dermatophyte from patients
   a) Examination of infected organisms.
   b) Isolation of infected organisms.

3- a) Screening of plant material for antifungal activity.
   b) Screening of different parts of the active plants for fungitoxicity.

4. Antifungal study of plant materials.

   a) Anifungal study of water extract against fungal organism.

   b) Anifungal study of solvent extracts against fungal organism.

   c) Anifungal study of oil extracted from active plants.

   d) Anti fungal activity of composite samples of selected plant materials.

   e) Anti fungal activity of plants solvent extracts on spore germination.

   f) Minimum inhibitory concentration of oils

   g) Study of fungicidal or fungistatic nature of oils.

5. Antifungal study of selected plant materials In-vivo.

   a) Sensitivity test of the oil on the human skin.

   b) Efficiency of the oil for the cure of infection.

6. Effect of plant materials on bio chemical parameters of blood in albino rats.

The above plan has been covered in the following chapters:

CHAPTER-1.Introduction: This chapter includes the introduction of the subject with its importance in the field of medicine as has already been described in the previous texts.

CHAPTER 2. Review of Literature - This chapter includes the review of the work done in the field with details of the available literature.

CHAPTER 3. Materials and methods - This chapter deals with the materials & methods used during the study of the present work.

CHAPTER 4. Observations & Results - This chapter deals with the observations & results obtained on the various experiments performed during the study.

CHAPTER 5. Summary & Discussion - This chapter deals with general summary of the entire work conducted and the result obtained has been discussed with those of the other workers.

CHAPTER 6. Bibliography

SECTION 2

REVIEW LITERATURE

Review Of Literature

Dermatophytoses consist a group of fungal infections, which involve keratinized tissue such as skin, hairs and nails. Dermatophytoses is the word used in order to differentiate it with Dermatophyte, which involve systemic mycosis. These Dermatophytes are fungi, which invade the keratinophilic tissue. Sabouraud 1910 classified them on the basis of clinical, cultural and microscopic characters. After words Langeron and Milo-chevitech (1930); Emmons (1934); George (1957); Ajello (1968) etc. have classified them on physiological, nutritional and morphological characteristics.

Several species of keratinophilic fungi has been isolated from soil. Most of these species are found to be parasitic on man and animals. Trichophyton rubrum, Trichophyton mentagrophyte and Epidermophyton floccosum has been found to cause infections of the foot like athlete's foot or ringworm. Trichophyton rubrum and Trichophyton mentagrophytes have also been found to causes ringworm of the nails, disease called Onychomycosis. Trichophyton rubrum is often found frequently associated with the glabrous skin in the groin and on the covered surface specially under tight clothing at the waist. Species of Trichophyton mentagrophyte may at times be zoophilic which may cause ringworm of the beard or barber's itch, the disease categorised as tinea barbae. Trichophyton rubrum may extend

over the body particularly to buttocks and waist area, the disease called dhobie itch. Microsporum gypseum has been reported to cause favus infection, which involves the scalp, and nails. Trichophyton mentagrophyte and Microsporum gypseum has also been reported on hairs and cause ringworm of the scalp.

White et. al., 1950 isolated Microsporum gypseum from soil by baiting, in which they burried wool in the form of baits in the soil. Vanbreuseghem (1952) used hairs as bait in the soil. Stockdale (1964) used 'hair bait' method to induce ascomycetons state of dermatophyte. Neel & Emmons (1939) isolated dermatophyte from 93 of 354 employees in an industrial plant. Fuentes (1956) isolated Microsporum nanum from a lesion of the scalp of a boy, from the body of an adult and also swine.

Dermatophyte has also been reported to be associated with cellulitis and granuloma. Andrew J. Velazyuez et. al., 2002 reported preseptal cellulitis on right eyelid by Trichophyton to a ten year old healthy boy.

Trichophyton has also been found to develop dermatophyte granuloma in HIV-I infected patient by Hadacek et. al., 1999; Voisard. 1999 reported a case of dermatophytic granuloma by Microsporum canis in a heart lung recipient. Other persons have reported many such cases also.

A number of person have tried to investigate the antifungal activity of plant material against dermatophytes but no compiled

work so far has been done. Only few antibiotic substances have been available in the market, which cause after effects and are thus not safe for general use. Some of these antibiotics have already been referred in the introductory chapter. Some of the work on plant material that has so far been done is being given in the following text.

Boecker 1939; Brigg 1942; Osborn 1943; Haddleson 1944; Lucas 1944 reported antimicrobial activity of higher plants.

Scott et. al., 1949 found that the leaves, stem and fruit skin of Musa sapientum inhibited the growth of Trichophyton mentagrophyte.

Lee and Chung, 1963 found inhibitory activity in ten medicinal plants against Trichophyton rubrum, Tetsuro et. al., 1967 found the nut shell of Juglans regia toxic against mentagrophyte. Swarts and Medrik (1968) found corn berry juice fungitoxic against eight dermatophytes. Ahmad et. al., 1973 found Juglans regia bark fungitoxic against Microsporum gypseum. Wollman et. al., 1973 found Pelargonium roseum aerial part active against Trichophyton mentagrophyte in a dilution of 1:1000 dilution. Tansey and Appleton 1975 found the bulbs of Allium sativum active against Microsporum gypseum and Trichophyton rubrum. Mukharya and Dahia 1977 found root of Plumbago species active against Microsporum gypseum. Amer et. al., 1980 found aqueous extract of Allium sativum toxic against Microsporum gypseum, Trichophyton mentagrophyte and Trichophyton

rubrum. They found complete healing of dermatomycosis during in-vivo trials. Chile et. al., 1981 found the entire plant of Vinca rosea active against Trichophyton rubrum in which the leaves showed maximum toxicity. Joshi & Bhatt (1983) found Chakramad durva, Haritake and Tulsi active against Microsporum and Trichophyton species. Singh (1984) showed fungitoxicity of seed extract of Embelia robusta and Saraca indica against Nannizzia fulva (-) and Nannizia fulva (+). Rao & Rao (1985) showed toxicity of Adenocalyma allicea leaf against Trichophyton mentagrophytes. Tripathi et. al., 1885 found pollen suspension of Xanthium strumarium active against Trichophyton mentagrophyte. Singh & Deshmukh (1985) found the bulb of liliaceae family active against Microsporum gypseum. Gupta (1988) found absolute toxicity of Acorus calamus leaves against Trichophyton mentagrophyte. Mishra et. al., (1988) found leaves of chrysantheum active against Trichophyton mentagrophyte. Tripathi et. al., 1990 found bark of Polyanthia active against Trichophyton mentagrophyte and Microsporum gypseum.

Dissalvo (1974) found that aqueous extract of Baccharia glutinoss leaves inhibited Trichophyton mentagrophyte and Trichophyton rubrum. Amer et. al., (1980) found aqueous extract of Allium sativum toxic against Microsporum gypseum, Trichophyton mentagrophyte and Trichophyton rubrum. Kim & Kwang (1980) found aqueous extract of Polygonum aviculare's leaves active against Trichophyton mentagrophyte. Dubey et. al., (1982b) found aqueous extract (1:1 w/v) of Citrus media and Erigeron bonariensis's leaves active against Trichophyton

mentagrophyte. Fuzellier et. al., 1982 found 5% that aqueous extract of Cassia alata's leaves showed antifungal activity against dermatophyte. Prasad et. al., 1982 found aqueous extract of Allium sativum at 1:10 concentration active against Microsporum canis. Which, they experimentally induced on rabbits. There was no side effect. Chun (1982) found aqueous extract of Allium sativum active against Trichophyton mentagrophyte and Trichophyton rubrum. Pandey et. al., 1983 found that aqueous extract (1:1 w/v) of Ageratum houstonianum showed absolute inhibition of mycelial growth of Microsporum gypseum. Tripathi et. al., 1983 showed toxicity of aqueous extract (1:1 w/v) of Iberis amara (seed) against Trichophyton mentagrophyte and Microsporum gypseum. Mall (1987) found aqueous extract of Eupatorium capillifolium and E. cannabinum leaves toxic against Microsporum gypseum, Trichophyton mentagrophyte and Trichophyton rubrum.

Hejtwankova et. al., 1973 showed that the some workers used several extract of the plant materials like alcoholic extracts of cupressaceae family such as Theyopsis dolabrate had the strongest activity against Trichophyton mentagrophyte and Trichophyton rubrum. Lalitha Kumari et. al., 1965 found alcoholic extract of Areca catechu more efficacious against Trichophyton rubrum than its aqueous extract. Tripathi et. al., (1978) found alcoholic extract of Inula racemosa's root toxic to Microsporum canis and Trichophyton mentagrophyte. Bhatt & Saxena (1979) found extract in chloroform, acetone & alcohol Anogoissus leiocarpa seed to show toxicity against Microsporum

gypseum. Kuntze et. al., 1979 found methanolic extract of Strobilanthes cusia (leaf) active against Trichophyton mentagrophyte. Khosa & Bhatia (1982) found alcoholic extract of Hypericum perforatum leaves toxic against some dermatophytes. Antonio et. al., 1986 found alcoholic extract of fifty six species of higher plants active against dermatophytes.

Many workers have shown that the toxicity varied from one family of flowering plant to the other. Singh, 1987 reported strong fungitoxic activity of the family Meliaceae. Dixit and Tripathi, 1987 found strong antifungal toxicity in caesalpinaceae. Gilliver, 1947; Dixit, 1978; Singh et. al., 1986 found strong fungicidal activity in Umbelliferae. Kishore et. al., 1981 found Verbinaceae to be actively fungicidal. The fungicidal activity not only varies from one family to the other if also varies from plant to plant of the same family as already shown in the previous text. Even the fungitoxic substance vary from one part of the plant to other part within the same plant. Ahmed, et. al., 1973 found Juglan regia bark fungitoxic against Microsporum gypseum. Tansey and Appleton, 1975 found bulbs of Allium sativum active against Microsporum gypseum and Trichophyton rubrum. Mukharya and Dahia, 1977 found roots of Plembago species active against Microsporum gypseum. Chile et. al., 1981 found the entire plant of Vinca rosea active against Trichophyton rubrum. In which leaves showed maxium activity. Similarly many workers like Singh, 1984, Rao & Rao, 1985; Tripathi, et. al., 1988; 1990 etc. have found different parts of different plants fungicidal against dermatophytes. Fungitoxicity varried from one

genus to the other within the same family and also from one species to the other within the same genus as observed by Tripathi, 1980; Asthana, 1984 etc. Thus plants containing fungitoxicity are scattered throughout the flowering plant and their activity is not related to their taxonomic position. Even some parts of the same plant are more toxic than its other parts.

Many person have studied the antifungal activity of the oils extracted from the plants such as Chaturvedi, 1979; Grover & Rao 1979; Asthana et. al., 1982; Renu et. al., 1985; Kishore, 1985; Mall, 1987 etc. These have used oil after obtaining them from the plants. While others like Sharma & Singh 1979, tested the commercial oils for their fungitoxicity.

Various persons have tried determining the minimum inhibitory concentration of essential oils. Such as Pandey et. al., 1983 found 100 ppm, concentration of Ageratum haustoniaum oil to be MIC concentration against Microsporum gypseum. Singh et. al., 1986 found 900ppm concentration of Trachysperrmum ammi against Trichophyton mentagrophyte.

Various persons have tried to findout the fungicidal or fungistatic nature of the oil. Pandey et. al., 1983 found Ageratum houstoniaum; Dubey 1981, Ocimum canum; Singh et. al., 1980 Cymbopogon martini oils to be fungistatic in nature while Pandey et. al., 1982; Kishore, 1985 exibited fungicidal nature of some oils.

In-vivo investigations on toxicity of plant constituents against dermatophytes have also been studied. Vichkanova and Kuznetsova 1967 carried out preliminary investigations on essential oil of Trapaeolum majus. They found it active against dermatophytes. Dixit et. al., 1990 found that essential oil of Eupatorium cannabinum and E. capillifolium in 1% ointment effective against experimentally induced ringworm on guinea pigs caused by Microsporum gypseum, Trichophyton mentagrophyte and Trichophyton rubrum.

Some workers have used plant materials to control various diseases. Steinhauer, 1993 found fungicidal activity of some compound from methanolic extracts of Azadiracta indica. Saraf et. al., 1991, 92 have found hair growth promoting activity of Tridex procumbens and have shown its hepatoprotective activity. Singh 1994 demonstrated the role of Azadiracta indica in common skin disorder in man. Kinungo et. al., 1992 have found it's effect on the prolongation of clotting time of rabbit.

Tinea capitis is a common clinical pattern of dermatophyte infection observed predominantly in children. It is becoming a Public health problem in some countries due to increased incidence. (Labato M.N.; Vugia D.J; Frienden I.J., 1997 : 1999) (Skerkev et. al., 1996). Its aetiology varies according to the regions of the world. With microsporum species being one of the predominant pathogen in Europe. Mostly in the Mediterranean and some central European countries. This dermatophyte is

zoophic and is mainly acquired from infected animals (e.g. pets) but may also be transtmitted by infected humans (Aly R. 1999).

Tinea capitis caused by Microsporum canis has been recognized as difficult to treat lower cure rates were achieved when compared with infections due to Trichophyton species (Krafchik, B. 1997). This may be in part due to the small spored ectothrix nature of the infection which makes it difficult for drugs to access.

Some reports have also indicated that Microsporum infections may require a longer duration of treatment to eradicate the infection compared with Trichophyton infections and that short-term terbinafine treatment is not associated with adequate cure rates (Hamm H. et. al., 1999), (Dragos V.; Lunder M. 1997). However, the optimal treatment duration for Microsporum related Tinea capitis has never been determined clearly.

Onychomycosis and Tinea pedis are the most common fungal infections encountered by the podiatric physician. Fungal infections of the toes are not uncommon. With prevalence estimates in the population ranging from 6.5% to 13.7%. (Gupta A.K., Jain H., Lynde C.W., 2000) and (Elewski Be Charif M.A., 1997). Dermatophytes cause the majority of toenail onychomycosis. Onychomycosis may be the most common nail disorder in adults Gupta, A.K. et. al., 1994.

Onychomycosis is a common infection in adults and accounts for 20% of all nail diseases. Approximately 30% of patients with dermatophyte infection on other parts of their body also have Tinea unguium, Onychomycosis affects toenails substantially more than fingernails. (Williams H.C., 1993).

The above review of literature shows that now-a-days dermatophytosis is spreading rapidly and is major concern the drugs available require a longer treatment and does not give adequate cure and the duration for treatment could not be properly determined. The potential for such treatment therefore lies in the Angiospermic plants as would be clear from the above text. Therefore, this work was undertaken to explore the possibility of a reliable treatment without any after effect.

SECTION 3

MATERIALS AND METHODS

Materials & Methods

During the course of study exercises mentioned in the introductory chapter were performed. The method used for the above exercises were as follows.

EXPERIMENT - 1 (A)

Collection of Plants and preparation of powdered materials :

Plants from the surrounding areas and neighboring places were collected during different seasons of the year. The plants were brought to the lab, washed thoroughly and than air dried at room temperature till constant weight. These plants materials were then grounded to powdered form of about 40 - 60 mesh size and than stored in plastic bottles for further use. About 64 plants belonging to 22 different families were collected. These were used for screening of antifungal activity against the test organisms since in the present study dried plant parts were found to loose their antifungal activity thus fresh parts of the plants were used.

EXPERIMENT –1 (B)

Preparation of water extract from plants powders :

After the performance of antifungal activity through the screening experiment out of 64 plants 3 plants species were selected for further test. Therefor water extract of plant parts

belonging to these plants were made. For this desired quantity of crushed plant 40-60 mesh size powdered material were taken in 500 ml. beaker, to which 100 ml. distilled water was added. These beakers were then kept on magnetic stirrer for 30 minutes. Then the material was allowed to settle and later filtered through whatman filter paper No. 1 under suction. The clear filtrate so obtained was used as distilled water extract. This extract, was used for testing antifungal activity through paper disk and for it's effect on the radial growth of the test fungus.

EXPERIMENT –1 (C)

Preparation of solvent extract from plant parts :

17 gms. of the plant materials of selected plants were kept 30 minutes separately in conical flask of 500 ml. capacity on magnetic stirrer in 100 ml. Acetone and Methanol solvents. The solvent extracts were obtained after filtration and evaporation under vacuum at room temperature. The solvent extracts so obtained were used fresh as and when prepared. For obtaining dilutions acetone was used.

EXPERIMENT - 1 (D)

Preparation of hot solvent extract by soxhlet :

17 gm. plant powdered materials of selected plants were taken for extraction of hot solvent extract in soxhlet apparatus. Solvents acteone and methanol were used. After complete exhaustion in soxhlet apparatus the extract was filtered by what

man filter paper No. 1 and the solvent was then evaporated under reduced pressure in a vacuum evaporator at room temperature. This extract was added with traces of tolumeme to prevent fungal growth and were kept in glass vials with stoppers in refrigerator for further experiments.

EXPERIMENT –1 (E)

Extraction of essential oil from plants materials :

Fresh plant materials of selected plants were collected and brought to the laboratory. These were washed thoroughly then subjected to steam distillation in Perkin's apparatus till clear distillate was obtained. The distillate was then saturated with Sodium chloride and extracted with petroleum ether in a separating funnel. The petroleum ether was then evaporated at 40^0 C on water bath. The essential oil so obtained was kept in vials with stoppers in refrigerator. The oils were diluted in acetone as and when required for studying the antifungal activity against the test organisms.

EXPERIMENT – 1 (F)

Preparation of composite samples :

In this experiment composite samples of solvent extracts from selected plants were prepared. For this solvent extract of different plants were mixed in different proportions. During this investigation three plant extracts were used. Out of these four types of composite samples were prepared, In one all the three

extract were mixed in equal proportion, in the second 1 ml. of 1 plant and half ml. of other two plants were mixed, Similarly two other samples were made in which the proportion varied accordingly.

EXPERIMENT – 2

Isolation of dermatophyte from patients :

Samples were collected from clinical patients who came to the O.P.D. of the dermatology section of M. L. B. medical college Jhansi, The patients suspected to be affected by dermatophytes [through Dr. Dinesh Govil Head & Proff. (Dermatologist)] were used for isolation of fungal materials. Depending upon the affected area isolation was conducted in the following manner.

When infection affected the skin the effected area was cleaned with cotton swab dipped in 70% alcohol. When dried the active edge of the lesion was scraped with a sterile scalpel and the material collected was transferred in a sterile petridish and brought to the lab for isolation of fungus involved.

When the infected area involved hairs the infected and damaged hair were taken out, the stumps of the infected hair were picked up with a forcep and transferred to sterile petridish for isolation of the fungus involved. Only the basal infected portions of the hair were used for isolation of dermatophyte in the petridish.

When nails were involved the superficial layer of the nail was scraped and discarded. The inner scraping were then taken

carefully in a sterile patridish and brought to the lab for isolation of the fungus. The specimens were not being stored in tightly stopper vials, moisture present would stimulate growth of contaminants and prevent isolation of dermatophytes.

EXPERIMENT - 2(A)

Examination of infected organisms :

The specimens collected from the medical college were brought to the lab where wet and transparent preparation was made to observe the fungus.

The scrapings were placed on the slide on which a few drops of 10% NaOH or KOH was kept to which a cover slip was put. This was heated on a flame slowly in such a manner that the slide remains bearable on the back of the hand. The slide was examined at once under the low power of the microscope with reduce light source. Young hyphe appeared as long undulant branching threads. Older hyphae may have septa which eventually break at the septa into barrel shaped or rounded arthrospores. The identity was made under the dry objective of the microscope. The most convenient stain for the quick diagnostic use was a mixture of NaOH and Parker's super blue black ink the amount of the ink had 20 parts of the ink to 80 parts of 10% hydroxide solution. For intense staining 20 parts of the ink and 20% hydroxide solution was used. The fungi took up the stain and the preparation improves upon standing for two to three hours. This staining was less intense then that of Lactophenol cotton blue. For nail examination they needed preliminary

digestion in 30% KOH in a test tube for three to four hours at 37° C followed by gentle crushing between slide and cover slip.

MEDIA PREPARATION (Emmons et. al., 1977)

SABOURAUD CYCLOHEXIMIDE-CHLORAMPHENICOL AGAR

Dextrose – 20gm.

Neo peptone- 10 gm.

Agar – 20 gm.

Chloramphenicol- 40 mg.

Cycloheximide – 500 mg.

Distilled water – 1000 ml.

Dextrose, peptone and agar were dissolved in 500 ml. distilled boiling water, 40 mg. of chloramphenicol was dissolved in 10ml. of 95% alcohol on flame. This was removed quickly from the heat and added to the media. 500 mg. Cycloheximide dissolved in 10 ml. of acetone was then added and the final volume was made up to 1000 ml. by adding distilled water. Media was then mixed properly and dispensed in 250 ml. conical flask in such a manner that they remain half filled. These were plugged and sterilized at 15 lbs pressure for 15 minute.

Cycloheximide reduced the rate of growth of many saprophytic fungi. It inhibits growth of the yeast forms of some dimorphic fungi when they incubated at 37^0c.

Chloramphenicol partially inhibits nocardia and other actionomycetes. Both antibiotics were omitted from media when they were not needed i.e. when pure cultures were handled during experimental work.

Fungus Preservation medium:

This medium was used to prevent pleomorphic variation in stock cultures of ringworm fungi :

Peptone	30 gm.
Agar	20 gm.
Water	1 Litre

The ingredients were dissolved, filtered through cotton gauze. Adjusted to pH 5.4, than distributed and autoclaved at 115^{0}C for 15 minutes.

EXPERIMENT -2 (B)

Isolation of infected organisms on agar media :

The infected samples brought from the O.P.D. patients of medical college Jhansi were used for isolation of fungal organisms on agar media. Sabouraud's agar media was used containing 0·05 mg/ml of chloramphenicol and pH adjusted to 6.5 to 7pH. Cycloheximide 0.1 to 0.4 mg./ml was added, to suppress the growth of saprophytic contaminating fungi. The cultures were incubated at 28^0 $\pm$ 2^{0}C, some dermatophytes grew and sporulated within 5 to 10 days but others required a longer time. In general cultures were

examined soon after sporulation began. The macro conidia were more easily seen in the first few days of sporulation then in old cultures.

EXPERIMENT – 3 (A)

Screening of plant material for antifungal activity:

The plant materials brought to the lab were screened for antifungal activity. For this purpose Distilled water extract of the plants were used. Distilled water extracts were prepared as described in experiment 1. (b). Sterilized and numbered filter paper disk were immersed in these extracts and were placed on seeded Sabouraud's agar medium with test organism. The extract soaked filter paper disk, which produced inhibitory effect, were noted, for further study. Experiments were run in triplicates.

EXPERIMENT -3 (B)

Screening of different part of the active plants for fungitoxicity:

Extracts from different parts of the plant that is stem/bark, leaf, inflorescence, fruit/seeds were tested for the toxicity against the test pathogen by paper disk method. So that the most suitable portion could be used for obtaining extracts for further use. For this experiment four most active plants were used. These were Cassia

tora, Vitex nigundo Ficus hispida and Trachysperrmum ammi.

EXPERIMENTS -4 (A)

Antifungal Study of water extract against fungal organisms:

The plant parts, which showed positive antifungal activity in the above experiment, were selected for further study on the radial growth of the test dermatophytes, to understand the behavior of plant extract and to further confirm the previous observations. For this experiment water extracts prepared in experiments 1 (b) were used. These were sterilized and were poured in sterilized petri dishes having sabouraud's medium at 40^0 c. The petri dishes were given a rotatory movement to mix the extract with the medium and the medium was allowed to solidify. The petri dishes were then inoculated with six mm. of the agar disk of the pathogen placed in the center. Control dishes were kept without any addition of plant extracts. Triplicates were taken for each plant extract for all the four pathogens.

The dishes were incubated at 28^0 C and the diameter of the growing colony was measured after every 24 hours up to 6 to 10 days. The diameter of slow growing colony were measured after every 48 hours and plotted against both cases with and without adding plant extract.

The percentage inhibition was calculated with the following formula.

$$\text{Percentage inhibition of mycelial growth} = \frac{dc - dt}{dc} \times 100$$

Where

dc = diameter of fungal colony under control condition

dt = diameter of fungal colony under treated condition

EXPERIMENT -4 (B)

Antifungal study of plants solvent extract :

This study was based on the agar diffusion technique in which an inhibition zone is developed if the fungal organism is susceptible to the diffusing solvent extracts. For this experiment glass cylinder plate technique was used. The plant extracts, which showed promising results in the previous experiments, were used.

The petri plates were poured with seeded Sabouraud's agar media. After jelling previously sterilized glass cylinders of uniform volume & size were fixed aseptically, by just flaming the bottom, along the periphery of the petri dishes. These cylinders were then filled with equal volume of solvent extracts. The inhibition zone produced was measured after 24 to 48 hours. The experiments were performed in triplicates and

the average inhibition zone produced by the solvent extracts against each of the pathogen were recorded.

EXPERIMENT -4 (C)

Antlfungal study of oils extracted from active plant parts:

This study was almost identical to the agar diffusion technique performed in the previous experiment. The preparation of plates with seeded sabouraud's agar medium was the same as described above. The only differences were that oils were used after mixing it with 1 ml. of acetone so that it could diffuse in the agar media.

EXPERIMENT -4 (D)

Antifungal activity of composite samples of selected plant materials :

Composite samples of the plant solvent extract were prepared by mixing different proportion of the different plant solvent extracts. For this experiment solvent extract of acetone was used since acetone extract was found to be better as compared to the methanol extract in the experiment 4 (b). Here plant extracts, which gave better result in experiment 4 (a) and 4 (b) were used for preparing composite samples. Four type of composite samples were prepared in which different plant solvent extract were mixed in the following proportion.

Composite sample	Cassia tora	Vitex negundo	Ficus hispida
1	1ml.	1ml.	1ml.
2	1ml.	0.5ml.	0.5ml.
3	0.5ml.	1ml.	0.5ml.
4	0.5ml.	0.5ml.	1ml.

These composite samples were taken in glass cylinders which were fixed on seeded agar of test fungus, Separate petri dishes were used for seeding Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum and Microsporum nanum. Experiments were run in triplicates.

EXPERIMENT -4 (E)

Antifungal activity of plants solvent extracts on spore germination:

For this experiment acetone solvent extract of Cassia tora, Vitex negundo and Ficus hispida were used to test their inhibitory effect on spore germination of Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum and Microsporum nanum. The extracts were taken in separate cavity slides to which equal quantities of spore suspensions of the above fungus were added. These were covered with a cover slip. The percentages of germinated spore were noted after 48 hours except in

Trichophyton rubrum where spore germination was observed after 96 hours.

EXPERIMENT -4 (F)

Minimum inhibitory concentration of oils :

In this experiment minimum inhibitory concentration of the oils were be identified which could be used for inhibition of growth of test fungus. For this the oils obtained from Cassia tora (immature pods), Vitex negundo (Leaves) Ficus hispida (Leaves) were used against test pathogen in concentrations of 5000, 2500, 1250, 625, 312 ppm. These dilutions were prepared by dissolving the requesting amount of oil in acetone, and then were placed in glass cylinder of uniform volume fixed on Sabouraud's agar medium.

EXPERIMENT -4 (G)

Study of fungicidal or fungistatic nature of oils :

This experiment was conducted to determine the fungicidal or fungistatic nature of the oils against the test pathogens this was determine as the per method followed by Garber Houston (1959). For this experiment dilutions of the oils were prepared as described in the experiment 4 (f) with sabouraud's dextrose agar medium in place of acetone. The control sets were run in which oil was replaced by the same amount of sterilized water. The

assay petri plates were inoculated with 6 mm fungal disk of the test organism from a 10 days old culture of the test pathogen and were incubated for 10 days. After this mycelial disk of the treated plates were taken out from the petri dishes. Washed thoroughly with sterilized water and re-inoculated to separate plate containing fresh sabouraud's agar medium. The presence and absence of mycelial growth in the treatment and re-inoculated disk were observed and recorded in the table XIII, XIV and XV.

EXPERIMENT - 5

Anti fungal Study of selected plant meterials In-vivo:

The active oils of Cassia tora and Vitex negundo were used for this experiment since these were found to be most effective in the previous experiments. These oils were separately mixed in transparent petroleum jelly and the paste so obtained were applied and given for application 2-3 times a day to the OPD patients at M.L.B. Medical College. The percentage recovery was noted after alternate days of application upto 23 days.

The in-vivo study of the oils obtained from active plant parts were conducted in two steps. In the first step the sensitivity test of the oil on human skin was observed and in the second step the efficiency of the oil for curing infection of the pathogenic fungus were studied.

EXPERIMENT -5 (A)

Sensitivity test of the oil on human skin :

In this experiment the study was done to find out the suitability of the oil by topical application on human skin was conducted. The test method as prescribed by Roxburg and Borrie 1973 was followed. Humans within the age group of 25 to 45 years were selected randomly. For each oil, group of 25 individuals were taken. An area of 4 x 1 cm on the lower side of the fore arm was washed with distill water followed by 70 % ethyl alcohol and allowed to dry for five minutes. 100 mg of ointment with 1% concentration of oil prepared in petroleum jelly was applied to the spot, and left as such. The application was repeated after every 24 hours for 10 days. The observations regarding various responses covering irritation, soothing effect, allergy etc. were recorded. Observations have been given in the Table XVI.

EXPERIMENT -5 (B)

Efficiency of the oil for the cure of infection :

For this experiment samples were collected from infected patients its direct examination and culture positive test were made. For direct examination the sample were placed on clean slide with one drop of 10 % KOH solution to dissolved the keratin and to separate the mycelium and conidia. The presence of mycelium or conidia of the test pathogen showed the establishment of

the disease. For these patients confirmation was also made by culture test. For this study Sabouraud's agar medium was prepared with cycloheximide and chloramphenicol. This medium was poured into presterilized petri plates and solidified the medium poured kept ready for cultural test. The cut pieces of the hair and skin scrapings were aseptically transferred on to the medium and incubated at 28° Cassia The cultures obtained were examined under the microscope for confirmation of the disease.

After confirmation, the in-vivo studies were made by the ointments of the oils. Prepared by mixing 1 ml of the oil into 100 gm of petroleum jelly and used twice in a day. The examination of treatment were made by placing the scrapping of skin or hair segments collected from the site of infection from patients on alternate days by the method already described above. The results were recorded in terms of percent culture recovery formula following Waheb et. al., 1982.

The data's obtained are being mentioned in the table XVII, XVIII and XIX.

EXPERIMENT - 6

Effect of plant materials on bio-chemical parameters of blood in albino rat :

The plant materials to be used for control of any disease should be such which should be non-allergic and should

not cause any change in the bio chemical parameters of the blood to which they would be directly exposed.

Therefore the present studies were planned on certain bio chemical parameters of blood in albino rat Rattus norvegicus after treatment with the active oils isolated from Cassia tora and Ficus hispida. Since long albino rats has been used in most of the laboratories as experimental animals for all such study through out the world. This rodent has served mankind in the study on various hereditary, physiological, bio-chemical and medicinal experiments, through which much knowledge have been gained. They are also sacrificed in many toxicological researches. Considering all these factors albino rat were considered to be the most suitable animal for the present study. In this study the effect of oils was studied on the change in the behavior of the rat, it's body weight, it's total Erythrocyte count, it's total Leucocyle count, it's hemoglobin contents, it's blood glucose and Serum cholesterol contents. For this study the following materials and methods were used.

Maintenance of rats:

For the present study albino rats were precured from the local animal supplier and then kept in clean ventilated cages measuring 45 x 30 x 30 cm these were kept over a tray for collecting fecal matters which were cleaned at regular intervals to avoid any infection. Cups for water and food were kept in each cage. The rats were fed with

cereals and were acclimatised to laboratory conditions for one month prior to the start of experimentation.

Application of Cassia tora & Ficus hispida ointment:

The experimental rat Rattus norvigicus was applied with the ointment prepared from the oils extracted from Cassia tora pods and Ficus hispida leaves in petroleum jelly. The hairs of an area approx. 2 cm^2 on either side of the central line of the back of the animal was removed with a clipper and then shaved with safety razor. In this area several pricks were made with sterilized niddle of the syringe and than the ointment of the extracted oil was applied. These areas were than covered with the piece of sterilized plastic sheet with the help of adhesive tape or bandage. In the control set no such application was made. In these experimental animals the application of ointment was repeated after every alternate day up to 21 days.

a) Behavior changes :

The behaviors of rats were noted after seven, fourteen and twenty day's treatment of the oils.

b) Food consumption :

Weighed amount of food was given to rats daily and it's consumption was measured by taken the difference

between the weight of food given and weight of the food left over. The controls were run separately without given any treatment to the rat. The food consumed was calculated as gm./rat/day.

c) Body weight :

The rats used in experimental set were weighed before and after treatment with plant oil mixed in petroleum jelly. The change in the body weight was assessed after 1, 7, 14, and 21 days treatment. Controls were run with these sets.

Collection and storage of blood :

The blood was collected from the veins and also from ventricles with the help of sterilized hypodermic syringe. For the storage of blood the following types of vials were used.

Double oxalate vials :

These were used for the estimation of morphological parameters of the blood. For this study 800 mg potassium oxalate and 200 mg ammonium oxalate were dissolved in 100ml of sterilized distilled water. In each vial four drops of this solution were taken and dried at $70^{o}C$ in an oven.

Fluoride Vial :

These were used for the estimation of blood glucose. For this experiment 2 gm of Sodium fluoride and 6 gm of Potassium oxalates were dissolved in 100 ml of sterilized distilled water. Four drops of this solution was taken in each vial and dried at $70^{O}C$ in an oven.

Separation of Serum :

For this sterilized glass centrifuge tubes were used for separation of serum. The blood samples were transferred to the centrifuge tubes, these were then left for about 45 minutes then centrifuged at 2500 rpm and the supernatant serum was separated by a rubber bulb pipette. The serum samples were stored in refrigerator. Both the whole blood and serum sample were used for the study of morphological and bio chemical parameters.

Morphological study :

(d) Total erythrocyte count and total leucocytes count :

In rats the leucocytes, thrombrocytes and erythrocyte are nucleated. Therefore it is not possible to discriminate between the leucocytes and erythrocyte by the method normally used in mammalian blood. Therefore for counting the leucocytes and erythrocyte following method described by Natt and Herrick (1952) was followed.

Preparation of diluents :

This was prepared by tabbing 3.88 gm Nacl, 2.50 gm Na_2So_4, 2.91 gm $Na_2HPO_4.12H_2O$, 0.25 gm KH_2PO_4, 7.5 cc. Fomaline (37%) and 0.10 gm. Methyl violet in distilled water in the order given above and then diluted to a total volume of 1 liter in a volumetric flask. The solution was kept over night and then filtered through whatman filter paper no, 42 the pH was adjusted to 7.3.

Procedure :

The blood was taken in R.B.C. pipette up to one mark and then the diluents was sucked up to 101 mark and allowed to stand for two minute. The blood sample was than shaken by given a rotatory movement to get equal cell distribution.

A drop of this diluted blood sample was placed on Naubauer counting chamber and covered with it's cover slip. The chamber was left undisturbed for two seconds, so that the cells could uniformly settle down. Then the slide was examined under high power objective. The R.B.Cassia were counted in the five square, of the center large square (four corner square and one center square) while the leucocytes were counted in all the 25 square of the center, large square containing 400 smaller squares. TEC and TLC were made directly from the same blood samples placed in the haemocytometer slide.

Calculation :

TEC (million/mm^2) = Total no. of erythrocytes x 5,000

TLC (thousand/ mm^3) = Total no. of leucocytes x 1000

(e) Haemoglobin Contents :

Hemoglobin content was determined by Shali's method suggested by Wintrobe (1981).

The graduated tube of haemoglobinometer pipette was rinsed with distilled water followed by 95% alcohol. After drying N/10 hydrochloride was sucked into it up to mark 2 cubic mm. Then fresh blood was sucked up to 20 cubic mm, mark. This was then transferred into graduated tube containing N/10 HCl. The content of the graduated tube were thoroughly mixed and allowed to stand for five minutes. Distilled water was then added drop by drop and stirred continuously with a glass rod, until the color of the content of graduated tube matched with that of the standard tube. The hemoglobin content was read directly on the graduated tube and expressed in gm/100 ml.

Blood Glucose :

This was estimated by O-loludine (span diagnostics kit code no. 25922).

Reagent in the kit :

Regent 1 - Trichloro acetic acid – 3%.

Regent 2 - Color reagent

Regent 3 - Glucose standard

Procedure:

0.2 ml of blood and 1.8 ml of trichloro acetic acid was taken in a test tube mix well and allowed to stand for 10 minutes at room temperature and then centrifuged. The clear supernatent obtained was added with the coloring reagent.

Glucose in the presence of acetic acid reacts with orthro toludine to give a blue green color complex, which was measured colorimetrically.

(f) Serum Cholesterol :

The serum cholesterol was estimated by the method described by Wybenga and Pileggi (1970).

This test in based on the fact that cholesterol react with ferric per chlorate, ethyl acetate and sulfuric acid and gives a lavender colored complex. Which is measured colorimetrically.

Reagent in the kit :

Reagent 1 - Cholesterol reagent

Reagent 2 - Cholesterol standard solution.

Procedure :

Three test tubes were taken and marked as test, standard and blank.

Test :

0.05ml of serum and 5 ml of cholesterol reagent was taken in the test tube marked test.

Standard :

0.05 ml of cholesterol standard solution and 5 ml of cholesterol reagent was taken in the tube marked standard.

Blank :

5ml of cholesterol reagent was taken in the test tube marked blank.

The content of each tube were mixed well and kept in the boiling water bath for ½ minute. Finally these were taken out cooled under running tap water. Reading of test and standard were taken colorimetrically against the blank by using green filter.

Calculation :

$$\text{Serum cholesterol mg/100 ml} = \frac{\text{Reading of the test}}{\text{Reading of the standard}} \times 200$$

SECTION 4

OBSERVATIONS RESULTS AND CONCLUSIONS

Observations & Results

This chapter deals with the observation made during the performance of various experiments. The observations made and results obtained are being given experiment wise.

Isolation of dermatophytes from patients :

Examination of infecting from organisms :

84 clinical patients of dermatophytes were made from the O.P.D. section of dermatology department of M.L.B. Medical College, Jhansi under the supervision of Dr. Dinesh Govil, Head and Prof. of the Department. These samples were obtained from different places of infection within body such as skin, hairs, nails etc. The clinical disease, effected area, symptoms and the number of patients involved have been given in the table I.

As per data recorded in the Table I Tinea corporis was found in 16 patients, Tinea cruris in 15, Tinea unguim in 14, Tinea manuum in 13, Tinea pedis in 14 and Tinea capitis in 12 patients among 84 patients. These samples were brought to the lab for isolation and then identification of the fungus involved.

TABLE – I

Isolates collected from patients at M.L.B. Medical College, Jhansi

S.N.	Clinical disease	Affected Area	Symptoms	No. of Patients
1	Tinea corporis	Stratum cornium layers of glabrous skin	Ringworm infection, ranging from scaling, to erythema to deep granulomata	16
2	Tinea cruris	Groin, perineum perianal	Sharply out lined raised, erythematous bordered with dry scaling center and pruritis or itching.	15
3	Tinea unguim	Finger nails and toenails	Superficial infection restricted to patches or pits on nail surfaces (white-onychomycosis) or chronic infection beneath nail plate, usually with hyperkeratosis and lifting or nail bed.	14
4	Tinea manuum	Interdigital and palmer surfaces of hand.	Similar to tinea corporis	13
5	Tinea pedis	Infection of feet particularly toe web & soles	Lesions very from mild and chronic scaling to acute inflammatory diseases with pustules	14
6	Tinea capitis	Scalp, eye brows and eyelashes	Sealy, erythematous lesions, hair loss, deep ulcer, rative eruptions; endothrix or ectothrix invasion of hair shaft.	12

Isolation of infecting organisms on Agar media :

The isolation from the above 84 samples collected from the dermatology department M.L.B. Medical College Jhansi, were made by transferring them sabouraud's agar media as per procedure described in materials and methods. The fungal organisms isolated from these samples has been mentioned in the table II.

Out of the 16 samples clinically thought to be of tinea corporis two samples were found negative for the occurrence of dermatophytes. From the rest of the 14 samples 13 were of Trichophyton rubrum and only one sample was of Microsporum nanuum.

Out of 15 samples from Tinea cruris one was negative, 10 samples were of Trichophyton mentagrophyte from, while 4 samples were of Trichophyton rubrum.

14 samples from Tinea unguium showed that 12 of them were of Trichophyton mentagrophyte while the rest two gave negative results.

From 13 samples from Tinea manuum, 11 gave positive results for the occurrence of Trichophyton mentagrophyte and the rest 2 gave the negative results.

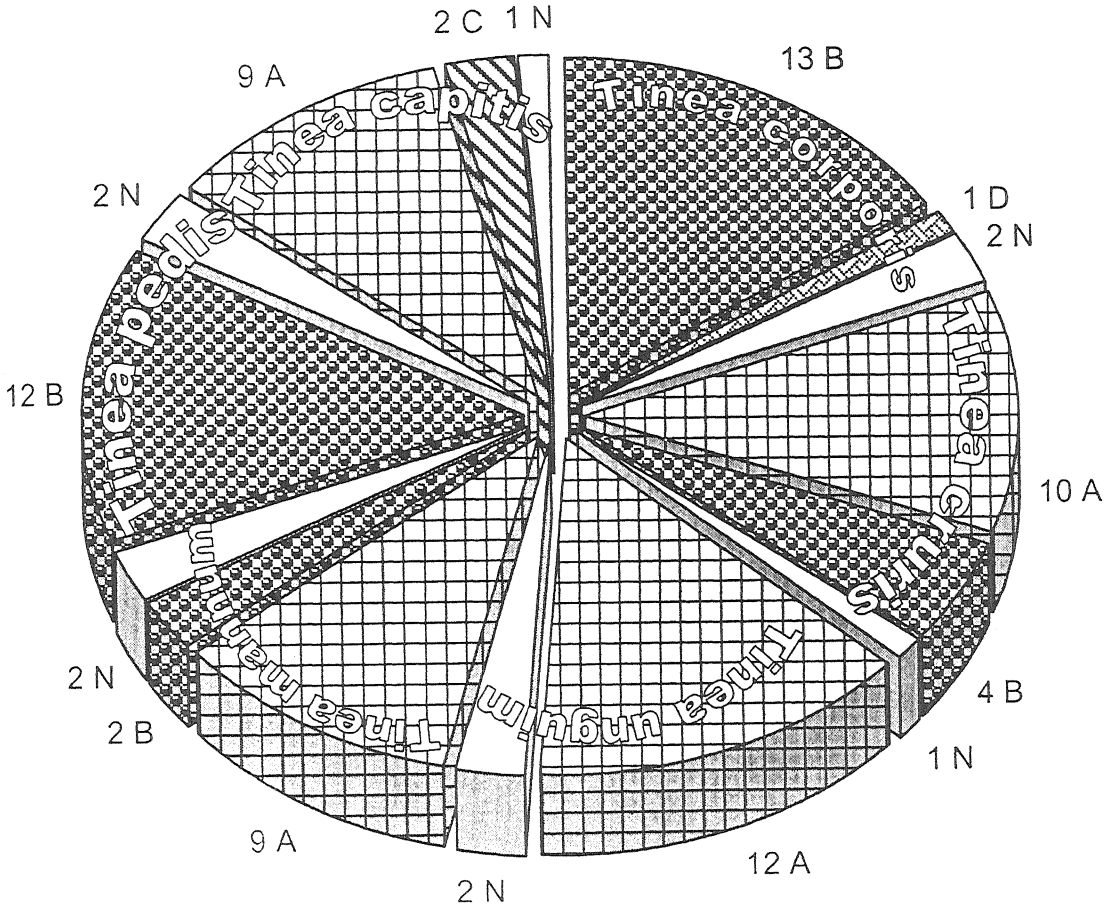
TABLE – II

Organisms isolated from samples of patients

S.N.	Clinical disease	No. of patients suspected	Name of organism	No. of Positive samples	No. of negative samples
1.	Tinea corporis	16	Trichophyton rubrum Microsporum nanum	13 01	02
2.	Tinea cruris	15	Trichophyton mentagrophyte Trichophyton rubrum	10 04	01
3.	Tinea unguium	14	Trichophyton mentagrophyte	12	02
4.	Tinea manuum	13	Trichophyton mentagrophyte Trichophyton rubrum	09 02	02
5.	Tinea pedis	14	Trichophyton rubrum	12	02
6.	Tinea capitis	12	Trichophyton mentagrophyte Microsporum gypseum	09 02	01

Fig. 1

ccurance of clinical diseases & organisms among patients at M.L.B. Medical College, Jhansi during random sampling



A = T. mentagrophyte
B = T. rubrum
C = M. gypseum
D = M. nanum
N = Negative sample

From the 14 samples from Tinea pedis, 12 were of Trichophyton rubrum while 2 were found negative.

Out of the 12 samples from Tinea capitis, 09 gave the positive occurrence of Trichophyton mentagrophyte and 02 of Microsporum gypseum while 01 was found negative.

From the over all result it can be observed that out of the 84 samples collected from almost the same number of patients, 10 didn't show any positive occurrence of the dermatophytes. It appears that these lesions might be due to some allergic response or of bacterial origin. Among from the rest of the 74 samples 31 were of Trichophyton rubrum, only 1 samples was of Microsporum nanum, 40 were of Trichophyton mentagrophyte while 02 samples were of Microsporum gypseum. Through these isolations four dermatophytes were isolated. The most abundant was Trichophyton mentagrophyte followed by Trichophyton rubrum then Microsporum gypseum and only one isolate was of Microsporum nanum.

This shows that Microsporum nanum is of very rare occurrence. The identification of the above dermatophytes was based on their morphological and cultural characteristics. Which was compared, with the standard monographs and authentic cultures obtained from All India Institute of Medical Science, New Delhi. The characteristics of these organism are described as under :

Trichophyton mentagrophyte (Emmonson, et. al., 1977) :

The species of Trichophytons are the most commonly isolated of all dermatophyte species from human ringworm infections. Their identity however, presents the most difficult of all the dermatophytes. Most of the species common to human infections fail to produce macroaleuriospores and there are limited physiologic tests available to assist in the differentiation of these species. Trichophyton is characterized by clavate macroconidia with smooth walls usually not exceeding 24μ in thickness and with zero to four cells of macroconidia.

Trichophyton mentagrophytes is nearly as common as Trichophyton rubrum as an etiology agent of dermatophytosis in human beings and is the most common agent of Tinea pedis. while being an important cause of most other forms of tinea as well. The colonies of Trichophyton mentagrophytes vary from white floccose colonies with no distinctive microscopic features except a few clavate microconida of cream coloured, yellowish or peach-coloured granular, flat colonies which bear spores freely. The morphologic features of these strains include clavate 3 to 4 septate macroconidia 6μ to 8μ x 20μ to 50μ in size; spherical or clavate microconidia; spirally coiled hyphae and nodular organs which are abortive ascogonia. The colors may include tan and reddish brown. The reverse pigmentation is equally variable and confusing. The colours may range from colourless or white, through various shade of brown to a red pigmentation. Trichophyton mentagrophytes fail to produce a red

pigment on corn meal dextrose agar. Whereas Trichophyton rubrum produce a red pigment consistently on it. Trichophyton mentagrophyton is further characterized by production of enzymes, which permit it to penetrate hair in-vitro by formation of deep narrow conical pits, and by production of urease. Most strains of Trichophyton rubrum either lack these enzymes or produce them slowly.

Macroconidia vary within the strain and from strain to strain from single celled spores 4μ to 8μ in size to 2μ to 5μ celled spores 8μ x 50μ in size. They may be most easily found in young cultures five to ten days old. Microconidia may be clavate and borne laterally on undifferentiated hyphae in floccose strains or nearly spherical on conidiophores in powdery or granular strains. The conidiophores may be once or twice branched to produce clusters of these sub spherical microconidia or microalerisopores. The short branches arising at almost right angles.

Trichophyton mentagrophyte falls within the "Small spored ectothrix" group, although most of the floccose strains of the type commonly isolated from Tinea pedis, do not spread to the scalp and do not invade hairs of the glabrous skin. The species includes strains, which do invade hair follicles and hairs and cause severe host reaction, which may be supportive expulsion of hairs and spontaneous termination.

ORGANISM -1

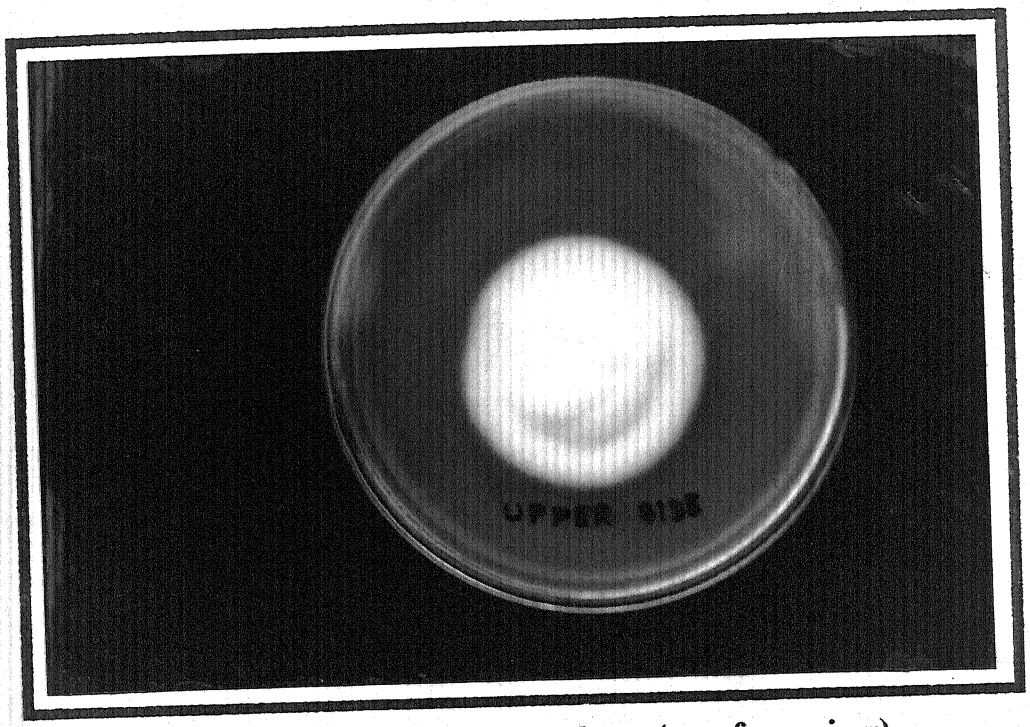


Plate - I Trichophyton mentagrophyte (surface view)

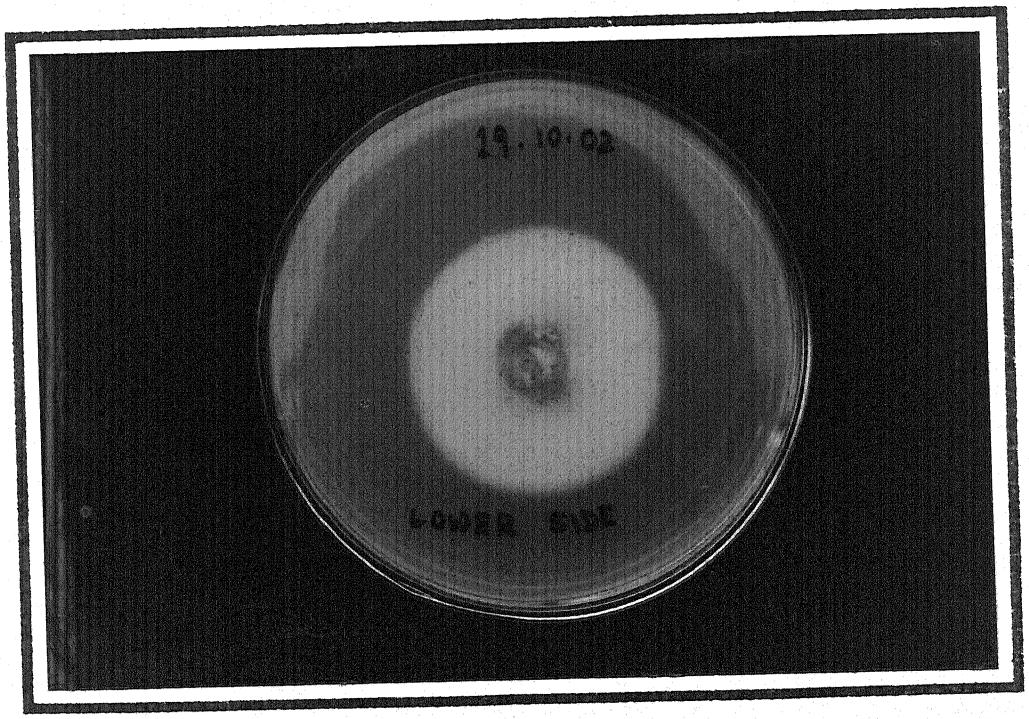


Plate - II Trichophyton mentagrophyte (reverse side)

Trichophyton rubrum (Emmonson, et. al., 1977) :

The anthropophilic dermatophyte Trichophyton rubrum is considered to be the most common cause of dermatophytosis in the United State and is normally isolated from Tinea corporis and Tinea pedis.

Trichophyton rubrum can be highly variable in morphology, but is normally seen on sabouraud's Agar medium as a slow glowing, heaped white to reddish floccose or velvety colony. The cherry red pigment is most apparent on the reverse side of the colony. The pigment usually develops after some weeks of growth but may be developed at all for some strains of Trichophyton rubrum. Particularly if the patient is on griseofulvin therapy. This red pigmentation can be more consistent at Trichophyton rubrum is grown on corn meal dextrose agar, while Trichophyton mentagrophyte fails to produce red pigment on this medium (Bocobo and Benham, 1949). On agar slant may appear first at the margin of a colony at the dry tip of the slant or at the center and in a concentric circle on the reverse side of a colony.

Macroconidia are typically long and narrow (4μ to 6μ x 15μ to 30μ) are sparse or lacking except on enriched media such as heart infusion tryptose agar. Microconidia are clavate (2μ to 3μ x 3μ x 5μ), borne laterally on undifferentiated hyphae or on simple lateral conidiophores. They may be almost sessile or on

ORGANISM -2

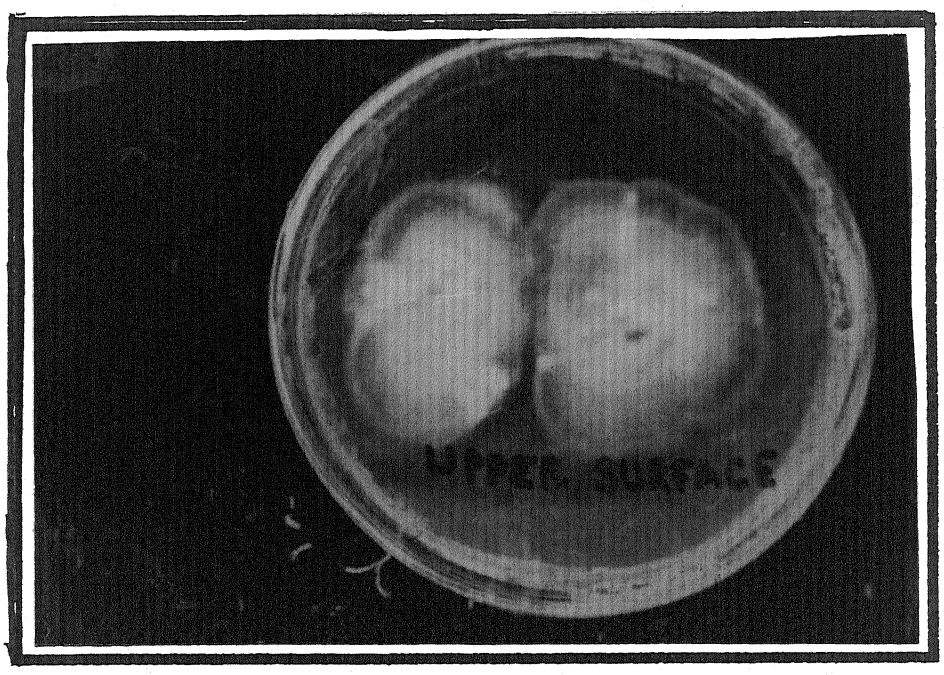


Plate - III Trichophyton rubrum (surface view)

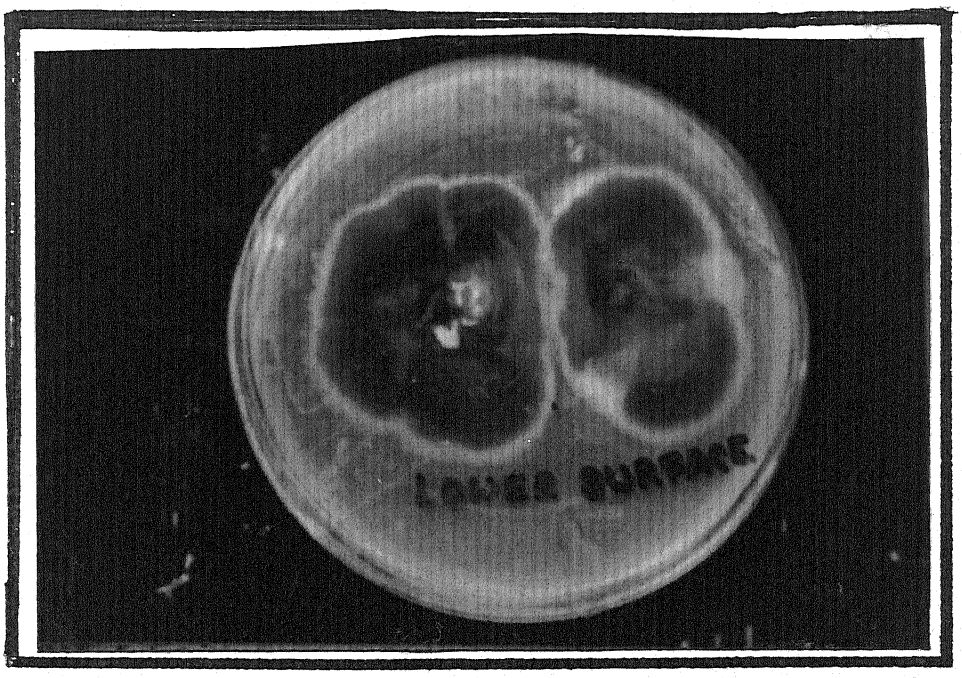


Plate - IV Trichophyton rubrum (reverse side)

ORGANISM -3

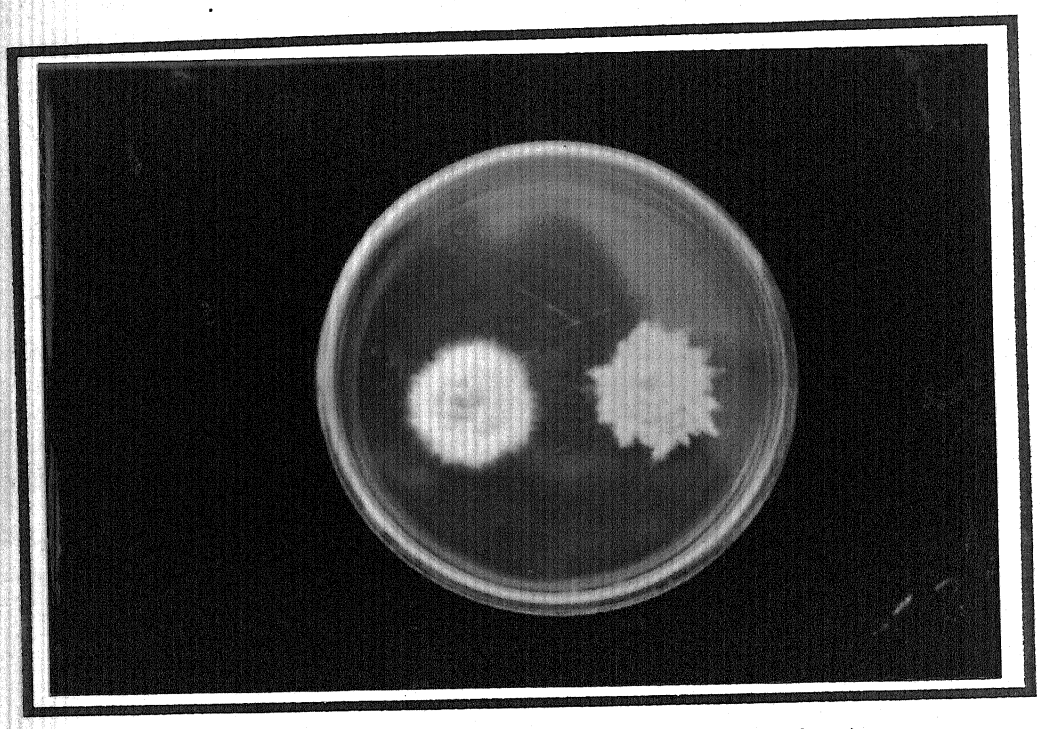


Plate - V Microsporum gypseum (surface view)

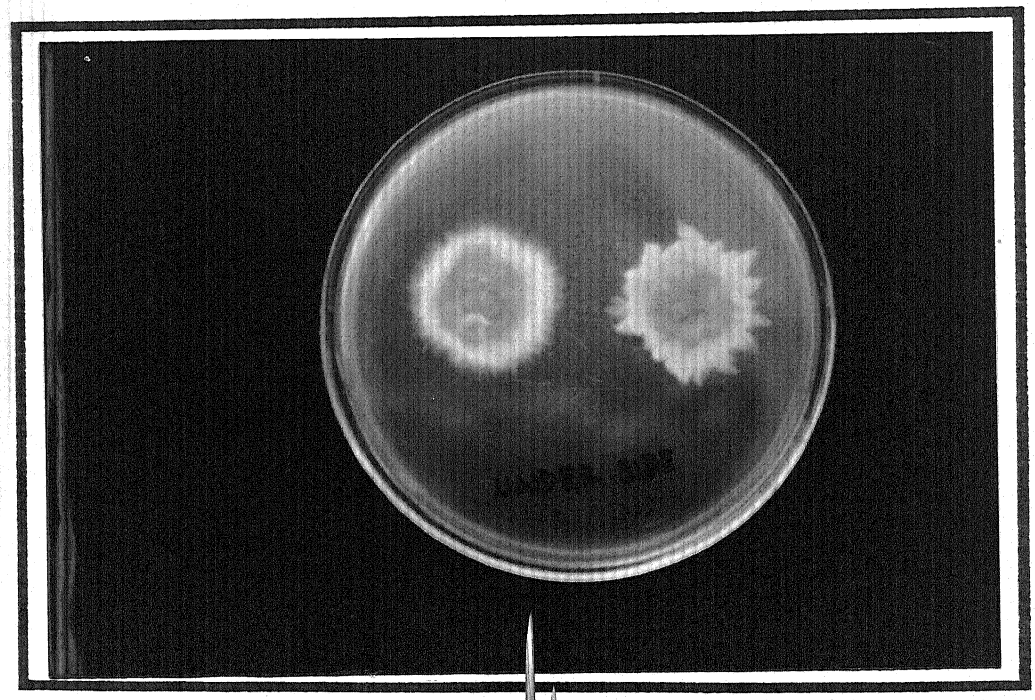


Plate - VI Microsporum gypseum (reverse side)

ORGANISM -4

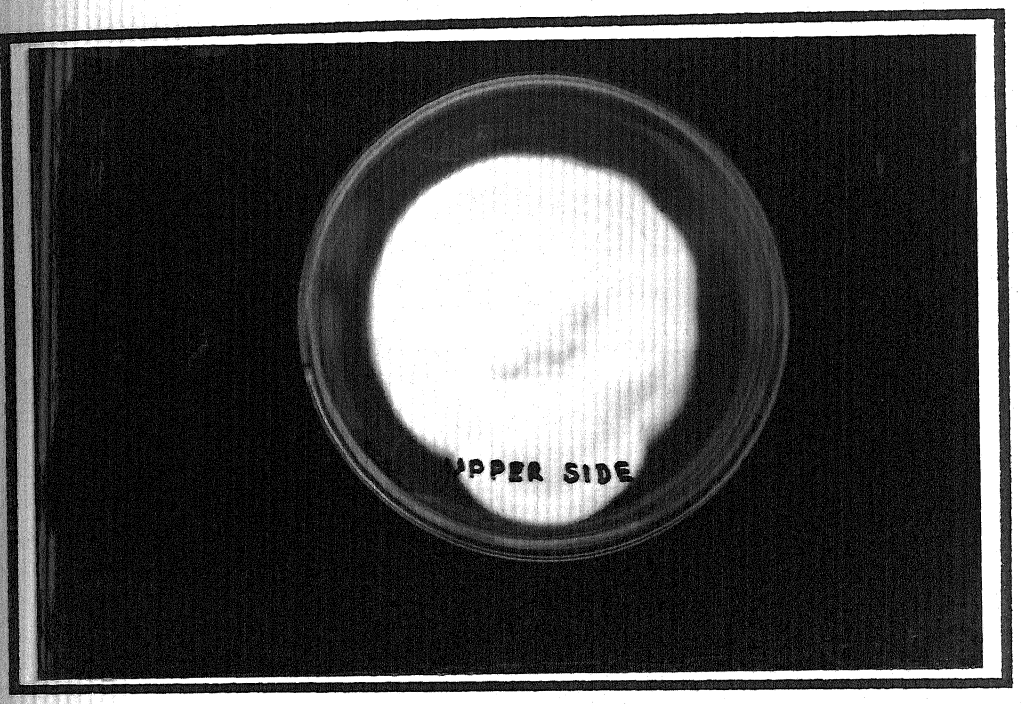


Plate - VII Microsporum nanum (surface view)

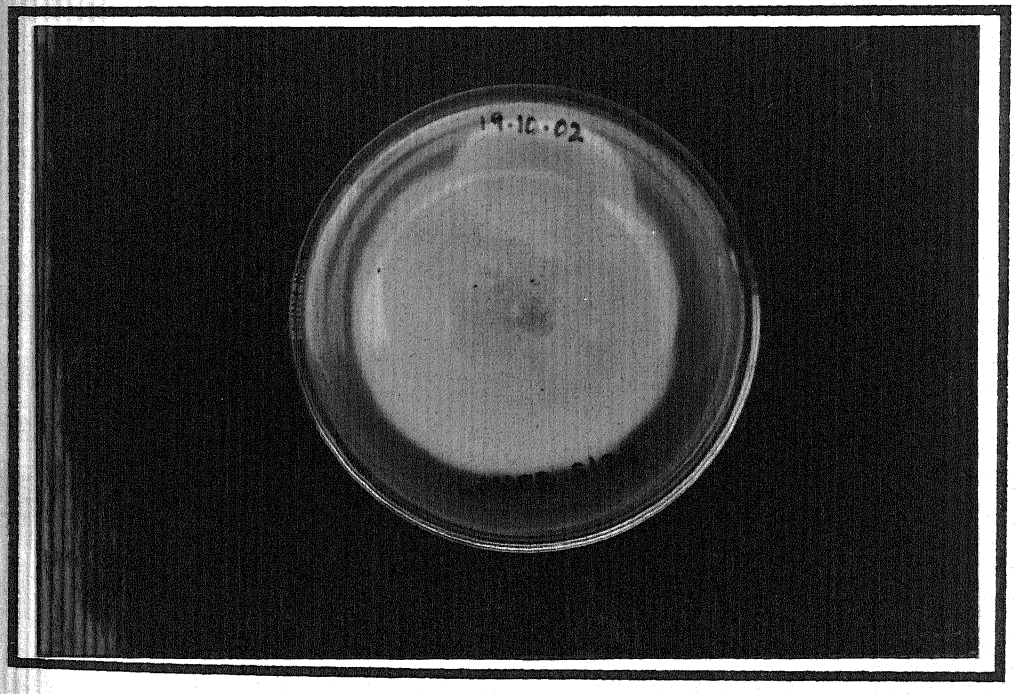


Plate - VIII Microsporum nanum (reverse side)

TABLE – III

Screening of plants for their antifungal activity

S.N	Plant	Tricho-phyton mentagrophyte	Tricho-phyton rubrum	Micro-sporum gypseum	Micro-sporum nanum
	Annonaceae				
1.	Annona squamosa L.	+ + +	+ +	+	-
2.	Polyalthia Suberosa Benthum & Hooker	+ +	+ +	+	-
3.	**Pappeveraceae** Argmone maxicana L.	+	-	+	+
4.	Papaver somniferum L.	+ + +	+ +	+	-
5.	**Brassicaceae** Brassica compestris L.	+ + +	+ +	+ +	-
6.	Iberis omara	+ + +	+ +	+ +	-
7.	**Caryophyllaceae**				
8.	Spergula arvensis –L	++	+	+	-
9.	Stellaria media –L	+	+	-	-
	Malvaceae				
10	Abelmoschus esculentus – L moench	++	++	-	-
11	Gossypium arborium -L	+	+	-	-
12	Hisbiscus rosa- sinensis -L	+++	++	+	-

13	Malvastrum coromandelinum -L Gareke	+	+	-	-
14	Sida cordifolia –L	+	+	-	-
	Linaceae				
15	Linum usitatissimum -L	+++	++	+	+
	Zygophyllaceae				
16	Tribulus terrestris –L	+++	+++	++	-
	Rutaceae				
17	Aegle marmelos -L	+++	+++	++	+
18	Citrus medica -L Var. lemon	++	++	+	-
19	Murrarya paniculata -L	+++	++	+	-
	Meliaceae				
20	Azadiracta indica	+++	++	-	+
	Papilionaceae				
21	Abrus precatorius -L	+++	++	+	-
22	Arachis hypogea -L	+	+	-	-
23	Butea monosperuma *taub.*	++	+	-	-
24	Cajanus cajan *Druce*	+	-	-	-
25	Dalbergia sissoo *Roxb*	+++	+	-	+
26	Dolichos lablab –L	+	-	-	-
27	Melilotus indica All	+++	++	+	-
28	Tephrosia purpurea	+++	++	+	-

	Caesalpinaceae				
29	Cassia tora	++++	+++	++	++
30	Bauhinia variegata	++	++	+	-
31	Cassia occidentalis	+	+	-	-
32	Saraca indica	+	-	-	-
33	Tamarindus indica	+	+	-	-
	Mimosaceae				
34	Pithecellobium dulce *Roxb*	++	++	-	+
	Rosaceae				
35	Rosa chinensis *Jacq*	+	-	-	-
	Myrtaceae				
36	Eucalyptus citridora *hooke*	+++	++	+	+
37	Psidiun guava	-	-	-	-
	Caricaceae				
38	Carica papaya -L	++	+	+	-
	Asteraceae				
39	Calendula officinalis	++	+	+	-
40	Eclipta prostrata	+++	++	+	-
41	Sonchus asper -L *hill*	+++	++	+	-
42	Tridex procumbens	-	-	-	-
43	Tagetes erecta	+++	++	++	+
	Apocynaceae				
44	Carissa carandas	++	+	-	-
45	Nerium indicum *mill*	++	-	+	-
46	Thevetia pruviana	+	-	-	-

	Asclepiadaceae				
47	Calotropis procera	++	+	+	+
	Solanaceae				
48	Capsicum annum	+	-	-	-
49	Dhatura alba	++	++	+	+
50	Clerodendrum indicum *kuntze*	+++	++	-	+
51	Lantana camera	+++	++	+	-
52	Vitex negundo	++++	+++	++	++
	Labiatae				
53	Ocimum basilicum	+++	+++	+	++
	Agavaceae				
54	Agave centala	+++	++	++	+
	Amaranthaceae				
55	Achyrantnes aspera	++	+	-	-
56	Amaranthus spinosus	+	-	-	-
	Cruciferae				
57	Nasturtium officinale	+++	++	++	++
	Euphorbiaceae				
58	Euphoribia hirta	++	-	-	-
59	Jatropa gossypifolia	+++	++	+	-
	Moraceae				
60	Ficus hispida	++++	+++	++	++++
	Zingiberaceae				
61	Curcuma domestica	+++	+	++	+
62	Zingiber officinalis	+++	+++	+	++

	Umbilliferae				
63	Trachysperrum ammi	++++	++	++	+
	Alliaceae				
64	Allium sativum	+++	+	++	+

+	Poor inhibition
+ +	Moderate inhibition
+ + +	Strong inhibition
+ + + +	Very strong inhibition
-	No inhibition

paper disk soaked in distilled water extract were used on seeded agar with the four test organisms isolated in the above experiments. Separate dish were taken for each fungal organism. The result obtained has been shown in the table III.

These plants were identified with the help of floras (Hooker 1872-1897; Duthie 1903-1929) as well as specimens present in the herbarium of Botany Department Biping Bihari Degree College, Jhansi. For such screening preference was given to those plants, which had literary record for use in skin diseases. The experiments were recorded in triplicates in terms of inhibitory zone. Data recorded are presented in the table III.

Total 64 plants were screened which belonged to 22 families, out of these Cassia tora, Carum copticum (Trachysperrmum ammi), Ficus hispida, Vitex nigundo showed maximum inhibitory effect on the four fungal organisms tested.

Rest of the plant species exhibited varying degree of toxicity. Therefore these plants were selected for further studies.

Some informations related to these antifungal plants described as under :

Cassia tora :

(Syn. C. Obtusifolia L.); Eng. Sickle Senna; Hindi – Pamaar, Chakunda (Caesalpinaiaceae)

A common herbaceous annual plant, occurring as a weed through out India. The pods are about 6 inch long and upto ¼ inch in diameter. The seeds contain glycoside and 5% fixed oil.

The leaves and seeds are useful in skin diseases Kirti & Basu (Wealth of India 1981) Cassia tora plant is useful in skin disease, such as Eczema. (Chunekar 1984).

Emodin is a glucoside, which present in seed of Cassia tora. That is like chrysophenic acid and 7.65% gum and cathortine present in leaves. Red pigment, minerals are also present in ash of whole plant (Sulphate, Phosphate, Sodium Iron, Potassium, Calcium and Magnesium) Sharma 1978.

Vitex negundo :

English Chinese chaste tree Hindi–Nirgundi, Samhalu (Verbenaceae). A shrub or small tree grown for reclamation of forestland. Leaves are considered as tonic also smoked for headache and applied to rheumatic swelling of joints. Used in several Ayurvedic preparation. Also possess insecticidal properties. Singh U.R. 1983. An extract of the leaves showed anticancer and antifungal activity is found to be effective the treatment of leprosy also with no tixic effect (Wealth of India).

Antifungal activity is found in vitex negundo. Vitex negundo leaves have a good antifungal activity (Chandra et. al; 1984). Leaves of Vitex negundo showed a good antifungal activity.

The leaves are aromatic, febrifuge, diuretic, emmenagoue, antihelmintic, expectorant and discutient. Paste of the tender leaves mixed with black pepper is given in fever. The leaf juice mixed with 'ghee' and black pepper is prescribed in rhenumatism. A mixture of leaf juice and Cow's urine is administered in enlargement of the speen (Kirtikar 1935).

Ficus hispida :

L.F.; Hindi – Kathumbar Konea – dumber (Moraceae)

A moderate sized tree or shrub distributed through out India. Leaves are large opposite 4-12 inch long and obovoid or turbinate, Fruits are 1 inch long, borne in pairs or clusters on leafbase often trailing branchlets. It occurs the flowers and fruits particularly through out the year.

The leaves are used for poulticing boils (Lauric, Loc. Cit; It Publ. Imp. Ogric. Bur., No. 10, 1947, 112; Burbill, I 1010) (Wealth of India – 1956).

The leaves contain crude protein, 12.36; ether extr., 2.75%, crude fibre 14: N-free extr., 58.88%; Total carbohydrate 71%; and total ash. 13% Lauric loc. Cit.

Trachyspermum ammi (L.) :

Sporague ex. Turrill (Syn. T. Copticum (L.) Link; Sison ammi L.; Carum Copticum (L.) Hiernj; English – Ammi, Lovage Hindi – Ajwain (Umbelliferae). A herb, cultivated on commercial scale in Uttar Pradesh, Madhya Pradesh, Andhra Pradesh and Bihar.

Fruits are used as spice, confectionary and beverages. Used in medicines for its antispasmodic, stimulant, tonic and carminative properties.

It is effective in sore throat and in bronchitis and forms an ingredient of cough mixture fruits also yield on essential oil which is main source of thymol. Oil is used as an antiseptic, carminative and insecticide. Fruits are also reported to have considerable antifungal activity against pathogenic fungi, Singh et. al., 1983. Carum copticum's fruits have a good antifungal activity, Chunekar, et. al; 1984.

Chemical composition : Essential oil 2 - 4%, Thymol 35 - 60%. Amount of Thymol should not be less than 40%. Thymol becomes solid after freezing. Rest is known as Thymene. Moisture 7.4%, Protein 17.1%, Fat 21.8%, Fibres 21.2%, Carbohydrale 24.6%, Minerals 7.9%, Ca – 1.525; Total P- 445, Fe 27.7, Na 56, K – 1.390, Thymine 0.21, Riboflamine 0.28, Nicotine acid 2.1 mg/gm, Karotin 71 mg/100 gm, Iodine 0.45 mg/ Kg. Except that Sugar, Tannin, Saponin, Glycoside, Flabone and steroid are also present.

Botanical descriptions of Antifungal plants :

The taxonomic classification of the all the four plants were done with the help of recognized herbaria of Duthie Hutchenson, Engler and Prantles as well as from the speciemens presereved in the herbarium of the Department of Botany. These plants are being introduced below in their respective families.

Caesalpiniaceae – Casia tora linn.(sickle sena)

Habit	A medium sized tree or shrub.
Root	Tap root, branched.
Stem	Erect, woody, branched, chlindrical, glaborous, Solid.
Leaf	Compound, alternate, paripinnate, pulvinus at the base, petiolate, stipulate (stipules minute, caducous); leaflets 4-8 pairs, ovate, entire, acute, glaborus, venation unicostate reticulate.
Inflorescence	Racemose, pendulous raceme.
Flower	Pedicillate (long pedicels), bracteate (bracts minute and caducous), bisexual, Zygomorphic, complete, hypogynous, yellow.
Calyx	Sepals 5, polysepalous, slightly petaloid, yellowish green, odd sepal anterior, quincunical aestivation.
Corolla	Petals 5, polypetalous, clawed, ascending imbricate aestivation, yellow.
Androecium	Stamens 10, polyandrous, unequal in length, 3 posterior stamens reduced to staminodes, remaining stamens fertile; anthers basifixed.
Gynoecium	Monocarpellary, ovary superior, unilocular, marginal, placentation; style short, stigma terminal, hairy.
Fruit	A legume.
Floral formula	Br, %, , K_5, C_5, A_{7+3}, $\underline{G_1}$.

Classification

Dicotyledons	Tap root system; leaves with reticulate venation; flowers pentamerous; embryo with two cotyledons.
Polypetalae	Perinath in two whorls; corolla polypetalous.
Calyciflorae	Flowers perigynous; sepals united, often adnate to ovary; petals uniseriate; stamens ten; thalamus cup-shaped; ovary inferior or semi-inferior.
Rosales	Alternate, stipulate leaves; flowers bisexual, zygomorphic; carpel solitary free in the bud; styles distinct.
Caesalpiniaceae	Bicompound leaves; pulvinus leaf base; flowers zygomorphic; corolla with ascending imbricate aestivation; monocarpellary unilocular ovary; marginal placement; stamens 10 in two whorls.

Antifungal plant-1

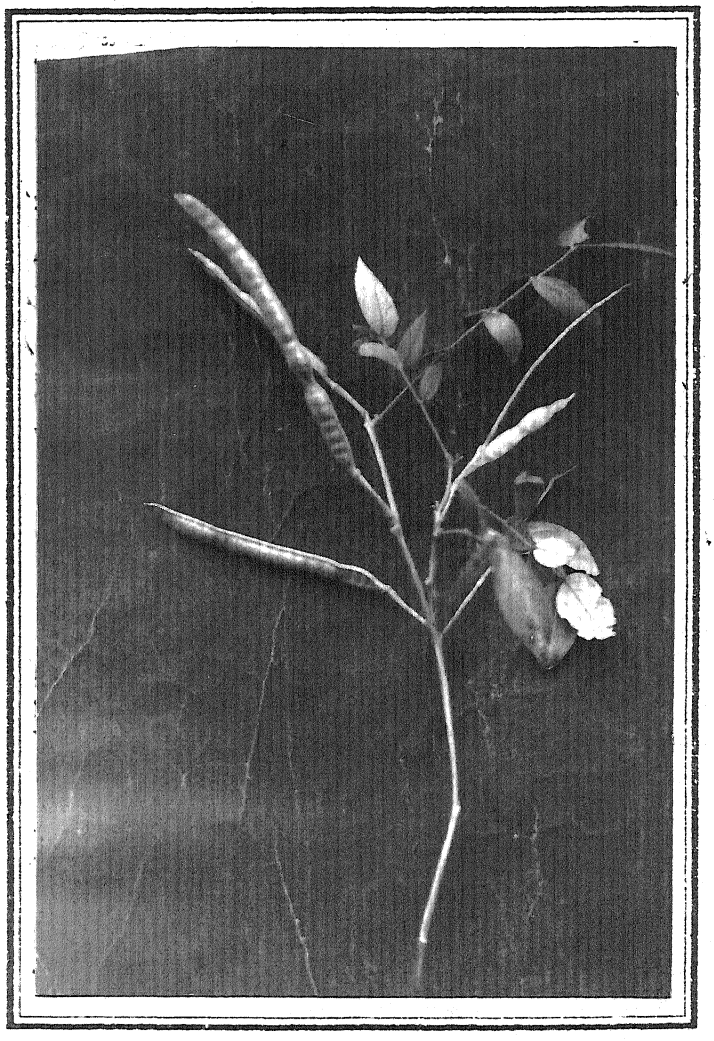


Plate IX *Cassia tora* twig with pod

Verbenaceae – Vitex negundo L.

Habit A shrub of 4 m height

Stem Cylindrical, branched stem.

Leaves Pinnately compound, digitately 3-5 foliolate, leaflets petiolate, oblanceolate, grey-pubescent below; non-aromatic.

Inflorescence A dichasial cyme with cincinnal tendency.

Flower Bracteate (bracts minute), pentamerous, bisexual, zygomorphic, complete, hypogynous.

Calyx Sepals 5, shortly 5- toothed, persistent, white-spotted without.

Corolla Petals 5, personate, upper lip 2-lobed, much shorter than the lower, lower lobes deflexed, much longer, corolla purple to violet.

Androecium Stamens 5, didynamous, exserted, eipipetalous; anthers are dithecous, introrse, dehiscing by longitudinal slits.

Gynoecium Bicarpellary, syncarpous, ovary imperfectly tetralocular, by false septum; ovary is superior, with a terminal style ends in a capitate stigma. There is one anatropous ovule in each loculus on axile placentation.

Fruit	Small, globose, drupe; endocarp a stony 4-celled, pyrene; seeds ovate-oblong, with a straight embryo.
Floral formula	Br, Ebrl, % , $K_{(5)}$, $C_{(5)}$, A_{2+2}, $G_{(4)}$
Classification	
Dicotyledons	Leaves with reticulate venation; flowers 4- or 5- merous.
Gynoecium	Petals united.
Bicarpellatae	Carpels two; flowers hypogynous, ovary superior.
Lamiales	Flowers zygomorphic; corolla bilabiate; stamens 4, didynamous or 2; ovary 2-4 locular; fruit drupe or carcerulus.
Labiatae	Stem quadrangular with decussate or whorled exstipulate leaves; inflorescence; verticillaster; gynoecium bilocular with 2 ovules in each loculus, sometimes tetralocular with one ovule in each loculus, style gynobasic; fruit carcerulus.

Antifungal plant-2

Plate X Vitex negundo twig

Moraceae – Ficus hispida linn

Habit	A cultivated hispid shrub or small tree
Root	Tap root, branched.
Stem	Aerial, erect, woody, branched, cylindrical, timber very light and soft.
Leaf	Large, coarse, roughly hairy, stalked ,opposite broadly ovate or ovate-oblong,4-9 inch,base rounded or slightly cordate or wedge shaped,margin toothed, short tip and abruptly pointed.
Inflorescence	Hypanthodium
Flower	Flowers minute, crowded on the inner surface of a hollow globose or ovoid receptacle, 3-bracteate at the base, less completely closed at the mouth, mixed with bracteoles, flowers of three kinds male, fertile female and imperfect barren female or gall flower.
Staminate or male flower	Usually placed near the mouth of the fig, short filament,perianth of 4 segments, thin, colo -urless, sometimes red or brown, stamen one.
Pistillate or Female flowers	Very minute,lateral,Perianth usually as in the male, ovary ovoid,distinct style,various stigma, ripe achenes, seed like, pale yellow.
Gall flowers	More resembling the fertile female flower, rudimentary style and stigma, empty ovary or

	contains the egg or pupa of an insect (never an ovule or seed).
Fruit	Syconus
Classification	
Dicotyledons	Two cotyledons; reticulate venation of leaves.
Monoclamydae	Flowers with simple perianth, sepaloid, some times altogether absent.
Unisexuals	Tepals sepaloid, much reduced or none; ovary ovoid.
Moraeceae	Leaves stipulate, hypanthodium inflorescence; fruits syconus.

Antifungal plant-3

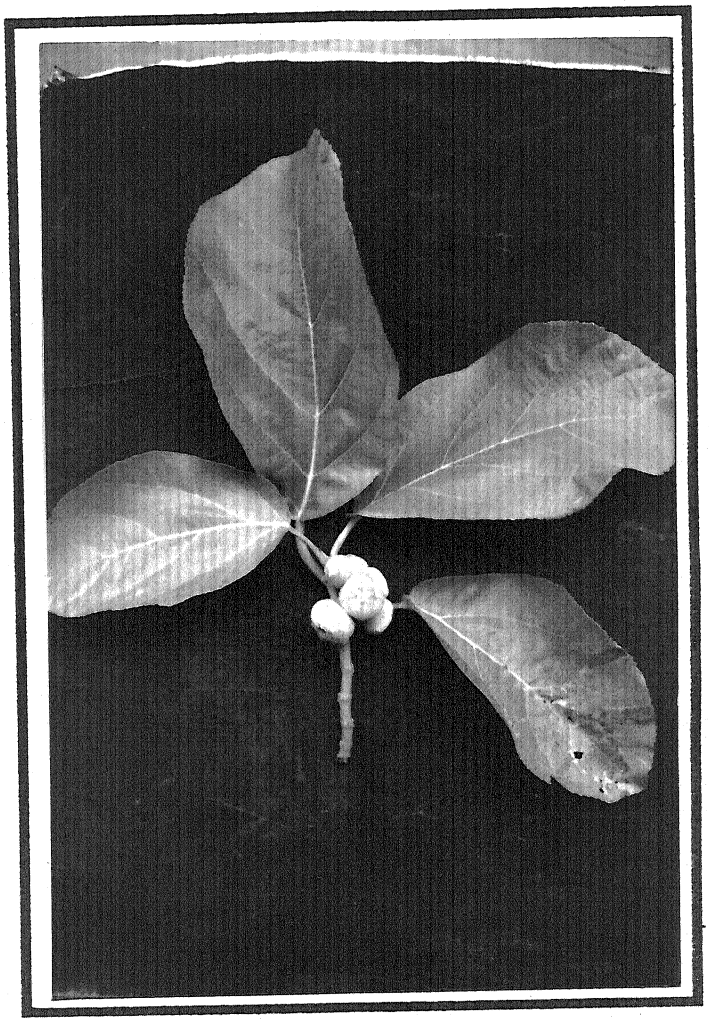


Plate XI Ficus hispida twig

Apiaceae (Umbelliferae) – **Trachyspermum ammi** (Linn.)

Sprague Syn. **Carum copticum** Benth & Hook

(lovage, ammi, *ajwain*)

Habit Annual aromatic cultivated herb.

Root Tap root, branched.

Stem Compound, 2, 3-4 pinnate or decompound, ultimate segments linear, segments wedge-shaped at the base, incised at the apex; leaves cauline, alternate, petiolate, exstipulate, leafbase sheathing.

Inflorescence Racemose, compound umbel, primary umbels with an involucre of bracts at the base and the secondary umbel with an involucel of bracteoles.

Flower Pedicellate, bracteate, bracteolate, bisexual, actinomorphic, complete, white, epigynous.

Calyx Sepals 5, gamosepalous, calyx tube adnate to the ovary wall.

Corolla Petals 5, polypetalous, retuse or emarginate (notched) yellow, valvate aestivation.

Androecium Stamens 5, polyandrous, alternipetalous, filaments, filaments long; anthers basifixed, introrse.

Gynoecium Bicarpellary, syncarpous, ovary inferior, bilocular, one ovule in each loculus, axile placentation, style extremely short, stigma bilobed, vittae (oil canals) present in ovary wall.

Fruit An ovoid cremocarp.

Floral formula Br, Brl, , , $K_{(5)}$, $C_{(5)}$, A_5, $G_{(2)}$

Classification

Dicotyledons Venation reticulate, flowers 4- or 5- merous.

Polypetalae Sepals, petals distinct; petals free.

Calyciflorae Flowers perigynous; sepals united, often adnate to ovary; petals uniseriate; stamens few or numerous, ovary inferior.

Umbellales Flowers regular, bisexual, in umbels, typically 4-5 lobed; stamens definite; ovary inferior, bilocular, ovules solitary and styles free; ovary inferior, bilocular, ovules solitary and styles free; leaves compound; resin or oil ducts present.

Umbelliferae (Apiaceae) : Herbs; stem hollow; much divided sheathing leaves; compound umbel; flower regular, bisexual; ovary inferior, honey-disc surrounding stigma; fruit cremocarp.

Antifungal plant-4

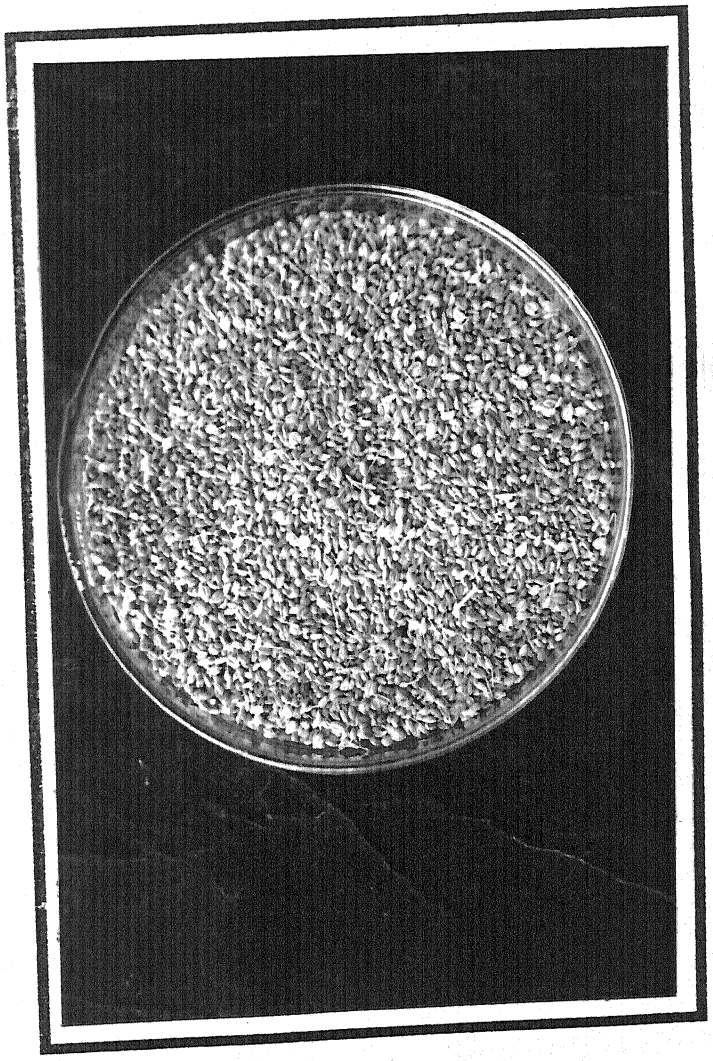


Plate XII Trachysperrum ammi seeds

Screening of different plant parts of the active plant for fungicidal activity :

For this experiment four plants were used Cassia tora, Vitex negundo, Ficus hispida and Trachyspermum ammi as will be evident from the finding obtained from the table IV. The most active portion of Cassia tora was the immature pods while in case of Vitex nigundo and Ficus hispida leaves showed maximum activity. While in Trachysperrum ammi seed showed the best toxicity against the test organisms. From this study pods of Cassia tora, leaves of Vitex negundo and Ficus hispida showed better activity than the seeds of Trachysperrum ammi. Therefore the former plants parts were selected for further studies.

Antifungal study of plant water extract against fungal organism:

For this study extracts of three most effective plants were used. These were Cassia tora extract, Vitex negundo extract and Ficus hispida extract. These extracts were mixed with Sabouraud's agar media and its effect on the radial, growth of four test fungal organism were studied. The radial growth were measured after every 24 hours for Trichophyton mentagrophyte, Microsporum gypseum and Microsporum nanum; while that of Trichophyton rubrum was measured after every 48 hours. Since it's growth was very slow. The growth of Microsporum nanum was found to be fastest growing, followed by Trichophyton mentagrophyte,

TABLE – IV

Fungitoxicity in different parts of active plants

Name of antifungal active plant	Part used	Trichophyton menta-grophyte	Trichoph-yton rubrum	Microspor-um gypseum	Microspor-um nanum
Cassia tora	Stem	+	+	-	-
	Leaf	+	-	-	+
	Flower	-	-	-	-
	Pod	+ + + +	+ + +	+ +	+
	Seed	+ +	+	+	-
Vitex negundo	Stem	+	+	+	+
	Leaf	+ + + +	+ + +	+	+
	Flower	+ +	+	-	-
Ficus hispida	Bark	+	-	+	+
	Leaf	+ + +	+ +	+ +	+ + + +
	Inflores-cence	+	+	-	+
Trachysperr um ammi	Stem	-	+	+	-
	Leaf	+	+	+ +	+
	Seeds	+ +	+ +	+ + +	+ +

\+ Poor inhibition

\+ + Moderate inhibition

\+ + + Strong inhibition

\+ + + + Very strong inhibition

\- No inhibition

Microsporum gypseum and Trichophyton rubrum. Controls were run side by side in which no plant extract was added.

Trichophyton mentagrophyte was found to be best inhibited by the immature pods extract of Cassia tora where the growth of only 13 mm. could take place after 144 hours. In presence of Vitex negundo extract it grew up to 17 mm. while in the presence of Ficus hispida it could grew up to 18 mm. in the same time as against control where a growth of 35 mm. was observed. This clearly shows that the inhibitory effect of Cassia tora was highest followed by Vitex negundo and Ficus hispida.

Microsporum gypseum was found to be best inhibited by Ficus hispida extract. This was followed by Vitex negundo and Cassia tora extracts. Where the growth of 21, 27 and 27 mm. respectively could be observed against 32 mm. in control after 144 hours.

Microsporum nanum was found to be the fastest growing organism amongst the four test organism. It's growth was best inhibited by Ficus hispida extract followed by Cassia tora & Vitex negundo extracts. In presence of Ficus hispida extract the growth of only 12mm. could be observed after 144 hours. While in the presence of Cassia tora 35mm. growth was observed and in presence of Vitex negundo 38mm. growth was observed during the same length of time.

Trichopyton rubrum grew up to 14 mm. in the presence of Ficus hispida extract as compared to 16 mm. in presence of Cassia tora

TABLE – V

Radial growth of test organisms in presence of Plant water extract

(Radial growth in mm.)

S. N.	Test Organism	Hours	Control	Cassia tora	Vitex negundo	Ficus hispida
1.	Trichophyton mentagrophyte	24	7	7	7	8
		48	12	7	8	8
		72	18	8	9	9
		96	23	9	9	12
		120	29	11	14	18
		144	35	13	17	18
2.	Microsporum gypseum	24	7	7	8	7
		48	11	8	8	9
		72	16	12	12	13
		96	21	16	15	17
		120	27	21	22	19
		144	32	27	27	21
3.	Microsporum canum	24	10	10	11	10
		48	15	13	12	10
		72	22	15	16	10
		96	32	20	22	11
		120	41	27	30	11
		144	50	35	38	12
4.	Trichophyton rubrum	48	6	6	6	6
		96	9	7	7	7
		144	13	7	8	8
		192	18	9	10	9
		240	22	12	13	11
		288	26	16	18	14

FIG. 5(A) Radial growth of Trichophyton rubrum in presence & absence of Cassia tora pod water extract

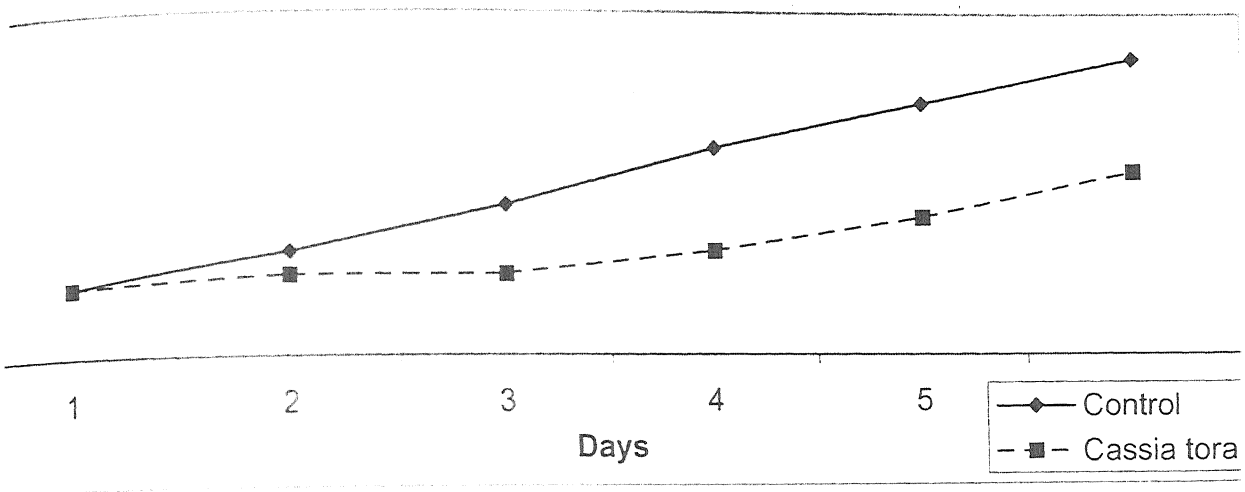


FIG. 5(B) Radial growth of Trichophyton rubrum in presence & absence of Vitex negundo leaves water extract

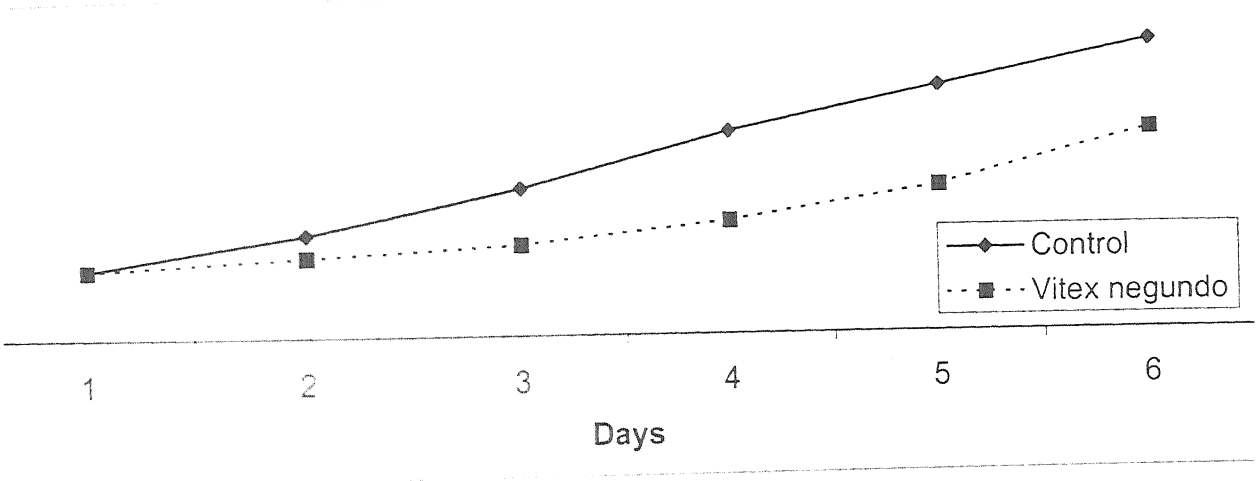


FIG. 5(C) Radial growth of Trichophyton rubrum in presence & absence of Ficus hispida leaves water extract

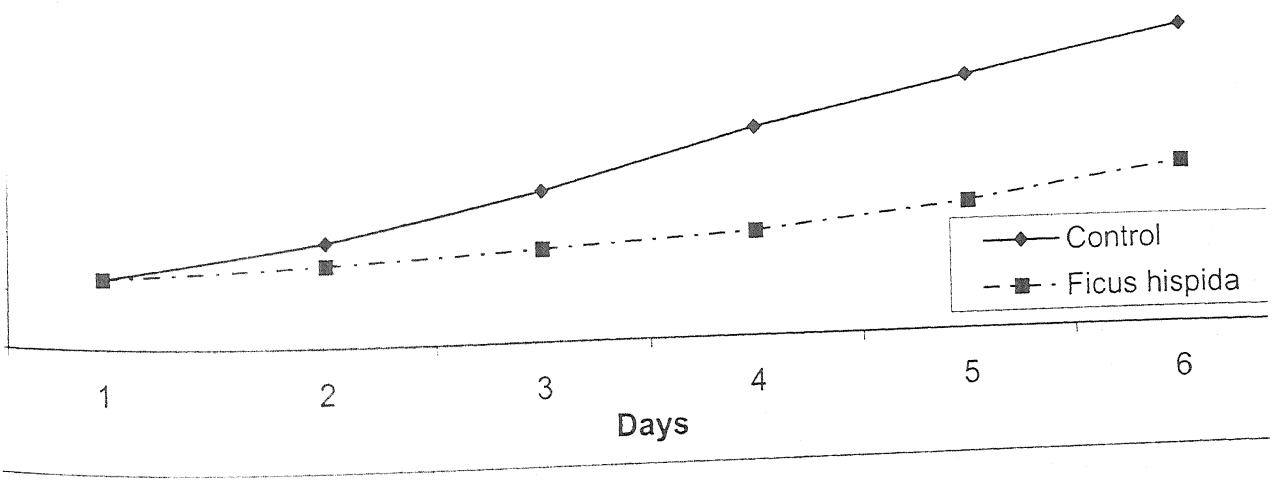


FIG. 3(A) Radial growth of Microsporum gypseum in presence & absence of Cassia tora pod water extract

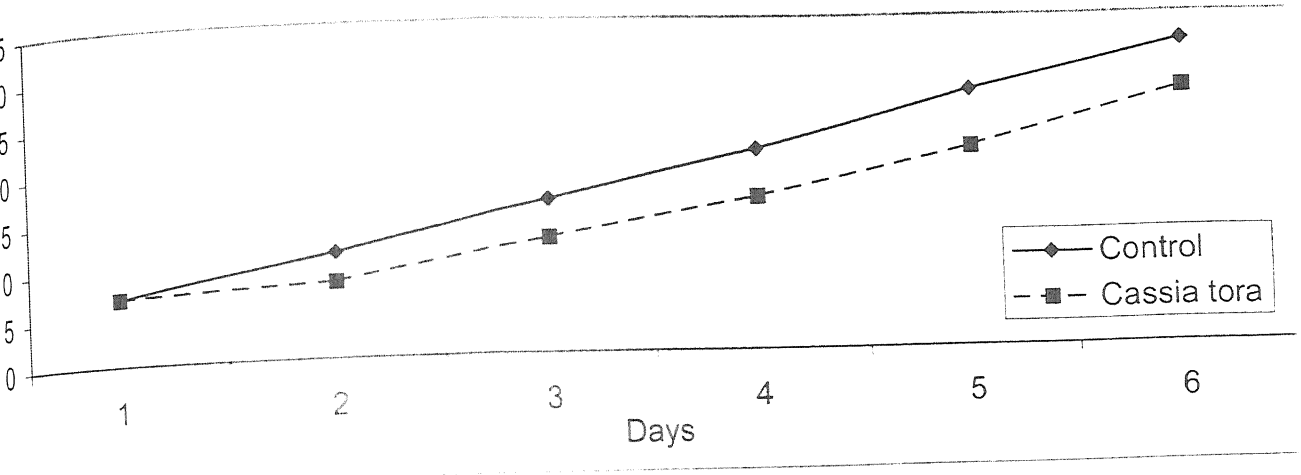


FIG. 3(B) Radial growth of Microsporum gypseum in presence & absence of Vitex negundo leaves water extract

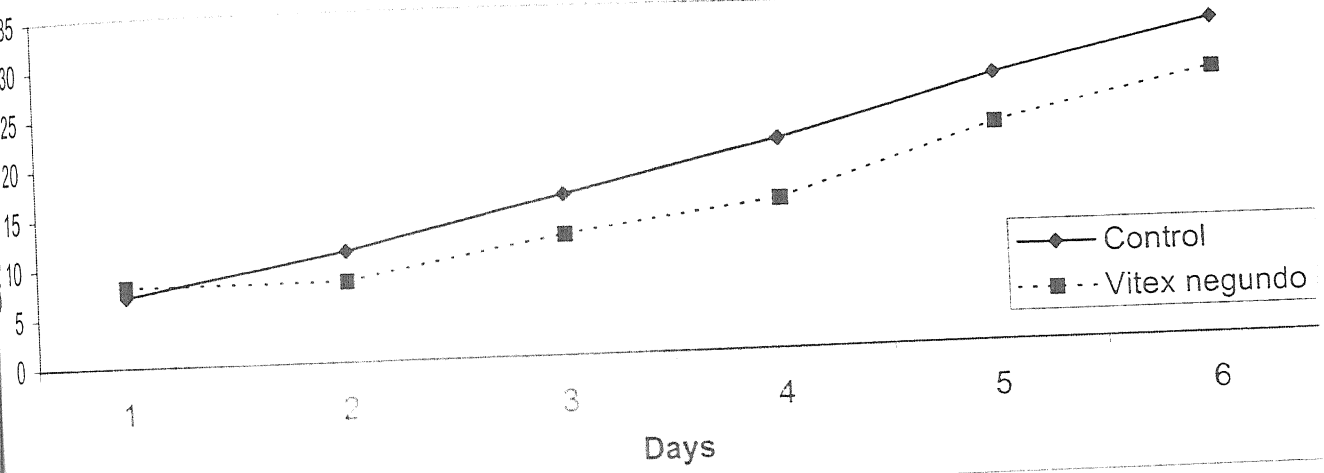


FIG. 3(C) Radial growth of Microsporum gypseum in presence & absence of Ficus hispida leaves water extract

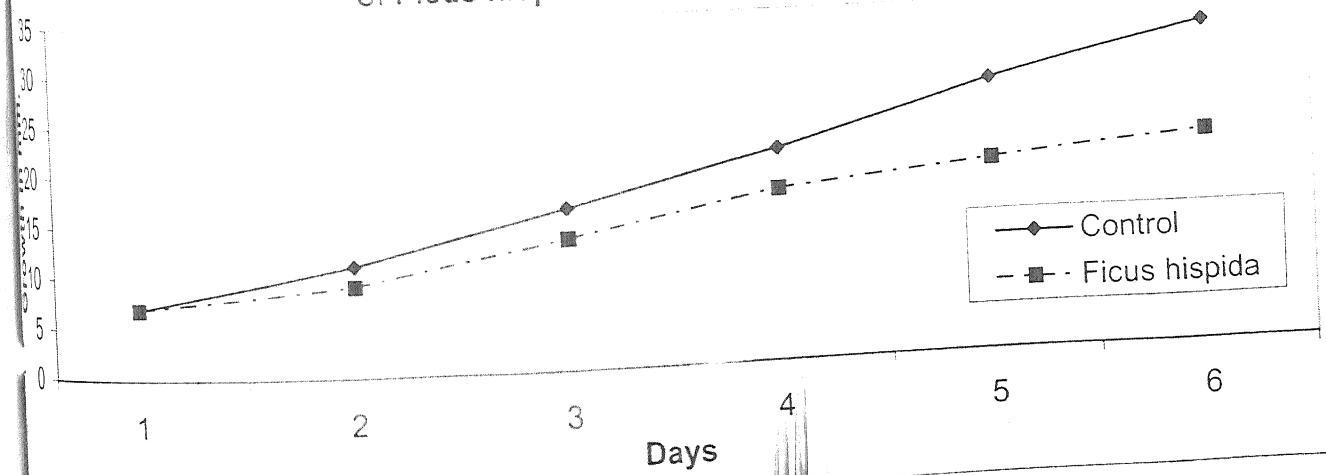


FIG. 4(A) Radial growth of Microsporum nanum in presence & absence of Cassia tora pod water extract

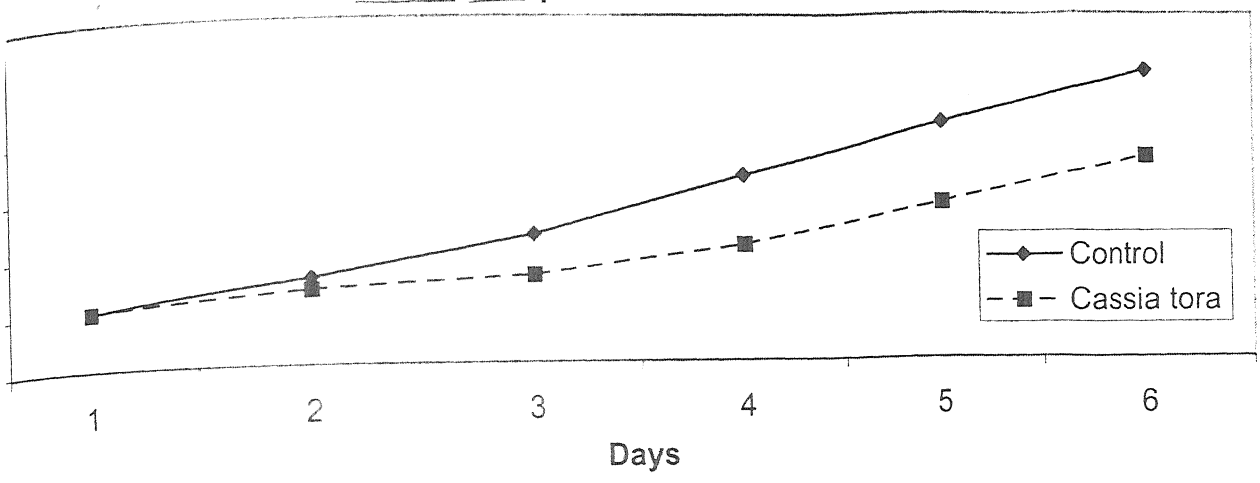


FIG. 4(B) Radial growth of Microsporum nanum in presence & absence of Vitex negundo leaves water extract

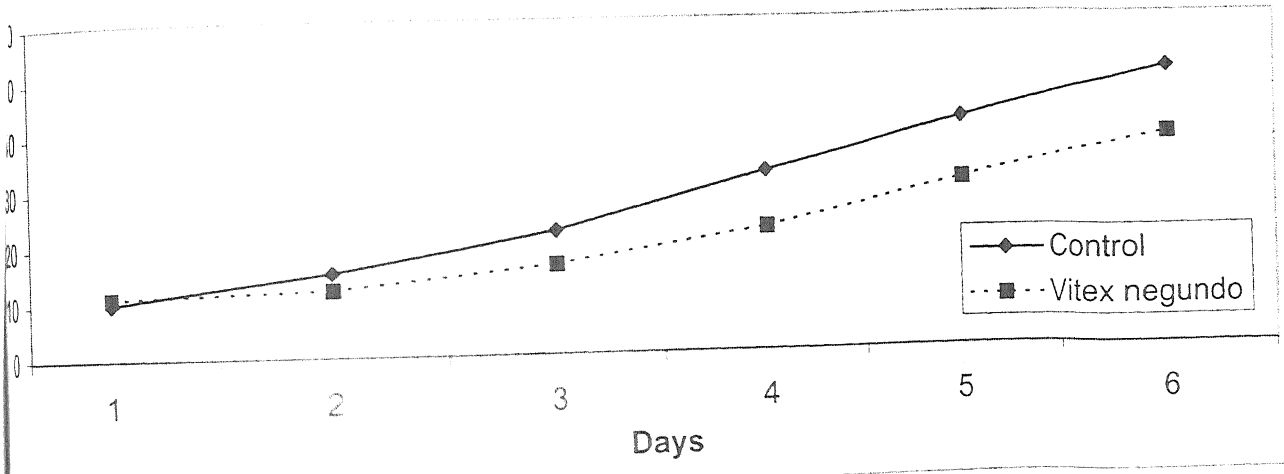


FIG. 4(C) Radial growth of Microsporum nanum in presence & absence of Ficus hybrida leaves water extract

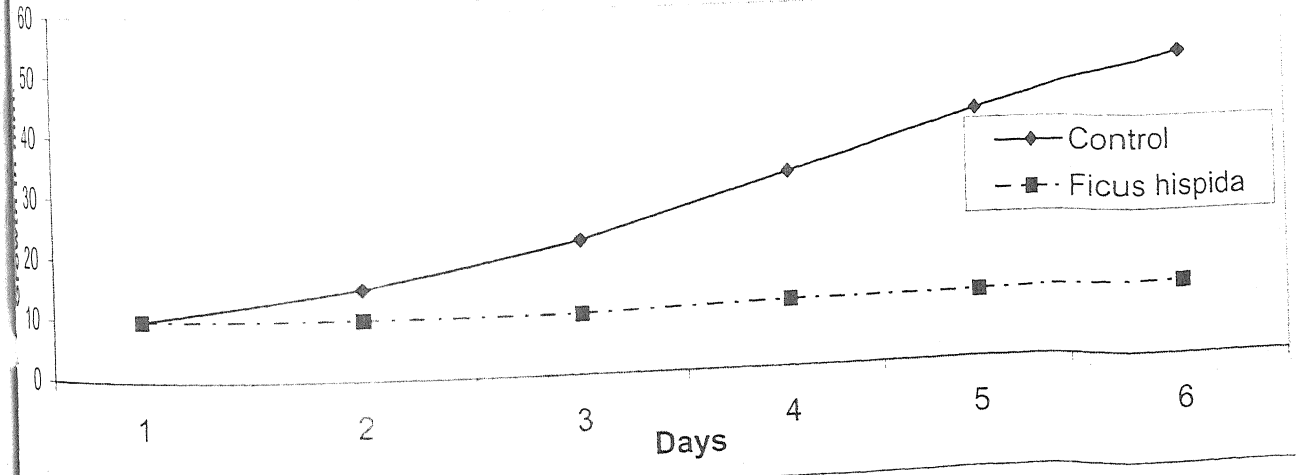
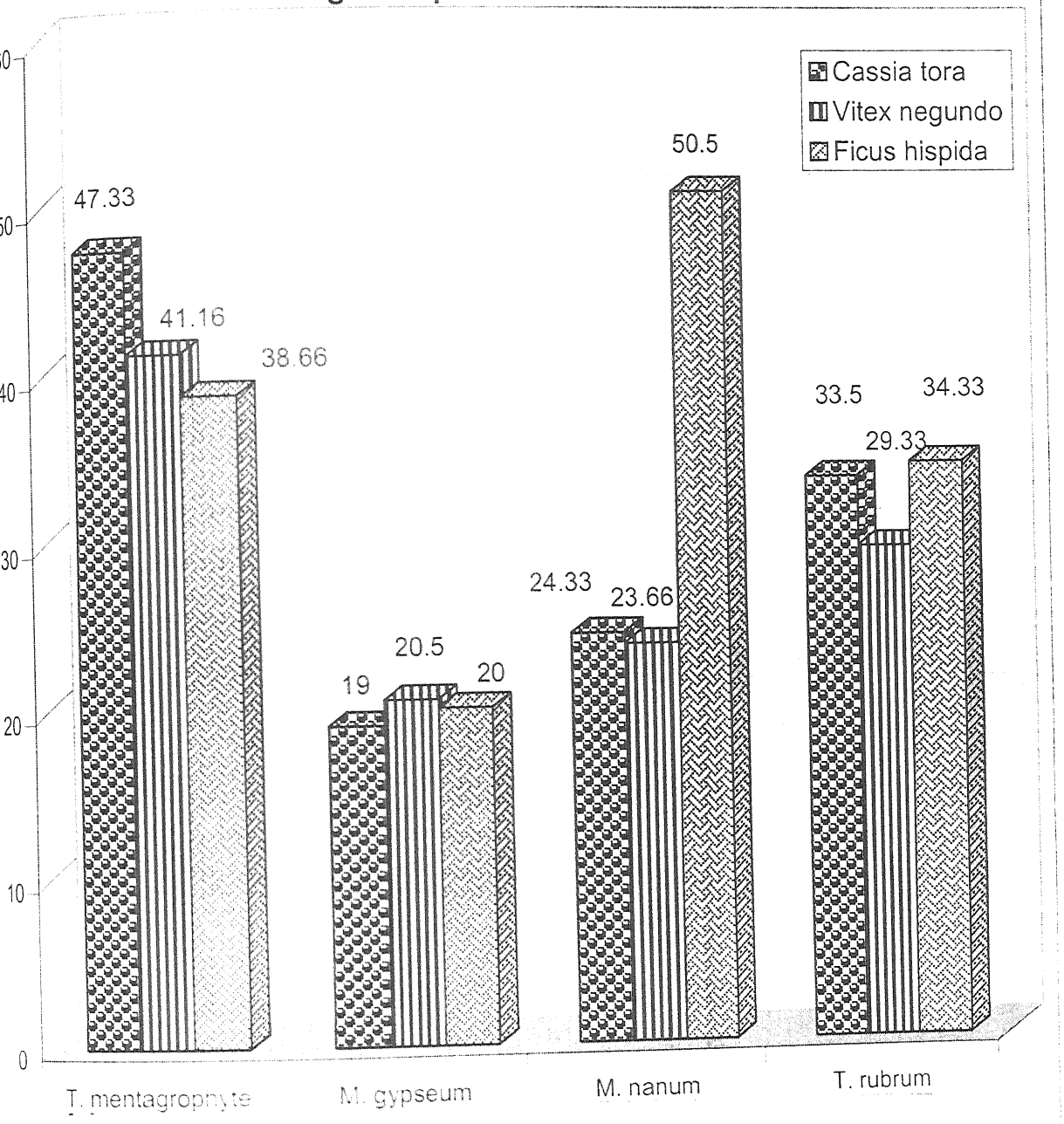


TABLE – VI

Percentage inhibition of test organisms against Plant water extract

S. N.	Test Organism	Hours	Cassia tora	Vitex negundo	Ficus hispida
1.	Trichophyton mentagrophyte	24	0	0	14
		48	42	33	33
		72	56	50	50
		96	61	61	48
		120	62	52	38
		144	63	51	49
2.	Microsporum gypseum	24	0	14	0
		48	27	27	18
		72	25	25	19
		96	24	29	19
		120	22	12	30
		144	16	16	34
3.	Microsporum audouinii	24	0	10	0
		48	13	20	33
		72	32	27	55
		96	37	34	66
		120	34	27	73
		144	30	24	76
4.	Trichophyton rubrum	48	0	0	0
		96	22	22	22
		144	46	38	38
		192	50	44	50
		240	45	41	50
		288	38	31	46

FIG. 6 Average % growth inhibition of test organism against plant extracts



and 18 mm. in presence of Vitex negundo extracts. This was slowest growing organism of test fungus which could grew upto 26 mm. in control plates after 288 hours.

The radial growth as observed has been recorded in the table IV.

The overall growth pattern can be best observed in terms of percentage inhibition as recorded in the table Vth. Highest percentage of inhibition was found in Cassia tora extract against Trichophyton mentagrophyte followed by Trichophyton rubrum, Microsporum nanum and Microsporum gypseum respectively Vitex negundo extract had maximum inhibitory effect against Trichophyton mentagrophyte followed by Trichophyton rubrum, Microsporum nanum and Microsporum gypseum respectively. Ficus hispida extract was most effective against Microsporum nanum followed by Trichophyton mentagrophyte, Trichophyton rubrum and Microsporum gypseum respectively.

Antifungal study of plants solvent extract against fungal organisms :

This study was conducted on the acetone and methanol extracts of Cassia tora. Vitex negundo and Ficus hispida against Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum and Microsporum nanum the solvent extract were used in glass cylinders fixed on seeded Sabouraud's agar media. The zone of inhibition obtained has been given in the table VII and VIII. From the data obtained it can be observed that Trichophyton mentagrophyte was best inhibited by the

acetone extract of Cassia tora which produced a inhibition zone of 19mm. while Vitex negunda acetone extract produce 16 mm. inhibition zone and Ficus hispida produced 14 mm. inhibition zone. Almost similar trend of inhibition was found with methanol extract of the three-plant material. However, the better result were of acetone extracts as compared to the methanol extracts.

Microsporum gypseum was best inhibited by Acetone extract of Ficus hispida followed by that of Cassia tora and then by Vitex negundo. The inhibition zone was less as compared to the other fungi. Since the inhibition zone was of 10 mm., 8 mm. and 7 mm. respectively. Methanol extract of the three plants were slightly less effective. It's Ficus hispida extract produced an inhibition zone of 9 mm. While it's Cassia tora and Vitex negundo extracts inhibition zone of 6.5 mm.

Microsporum nanum was best inhibited by Ficus hispida acetone extract where inhibition zone of 18 mm. was produced followed by Cassia tora acetone extract with 14 mm inhibition zone and Vitex negundo with 13 mm inhibition zone the result were almost similar however with lesser degree in Methanol extract.

Trichophyton rubrum was very slow growing fungus therefore, distinct inhibition zone could be observed after 21 days. The best results were obtained in the solvent extracts of Cassia tora followed by Ficus hispida and then Vitex negundo. All the three

TABLE – VII

Inhibitory effect of test organisms against plants acetone extract

(Inhibition zone in mm.)

Organisms	Observation Days	Cassia tora	Vitex negundo	Ficus hispida
Trichophyton mentagrophyte	14	19	16	14
Microsporum gypseum	14	8	7	10
Microsporum nanum	14	14	13	18
Trichophyton rubrum	21	16	14	15

FIG. 7 Inhibitory effect (mm) of Test organisms against plant acetone extract

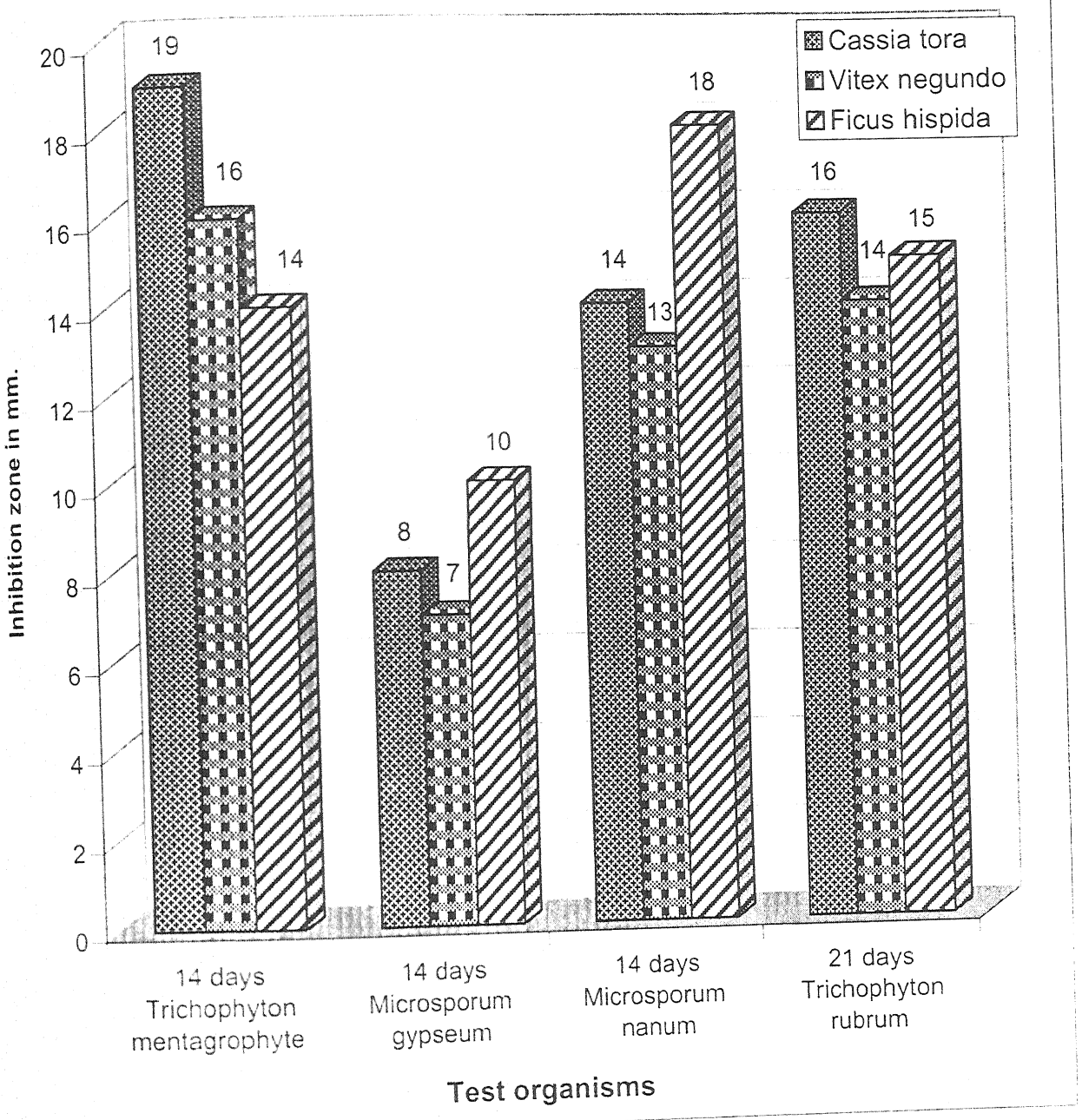


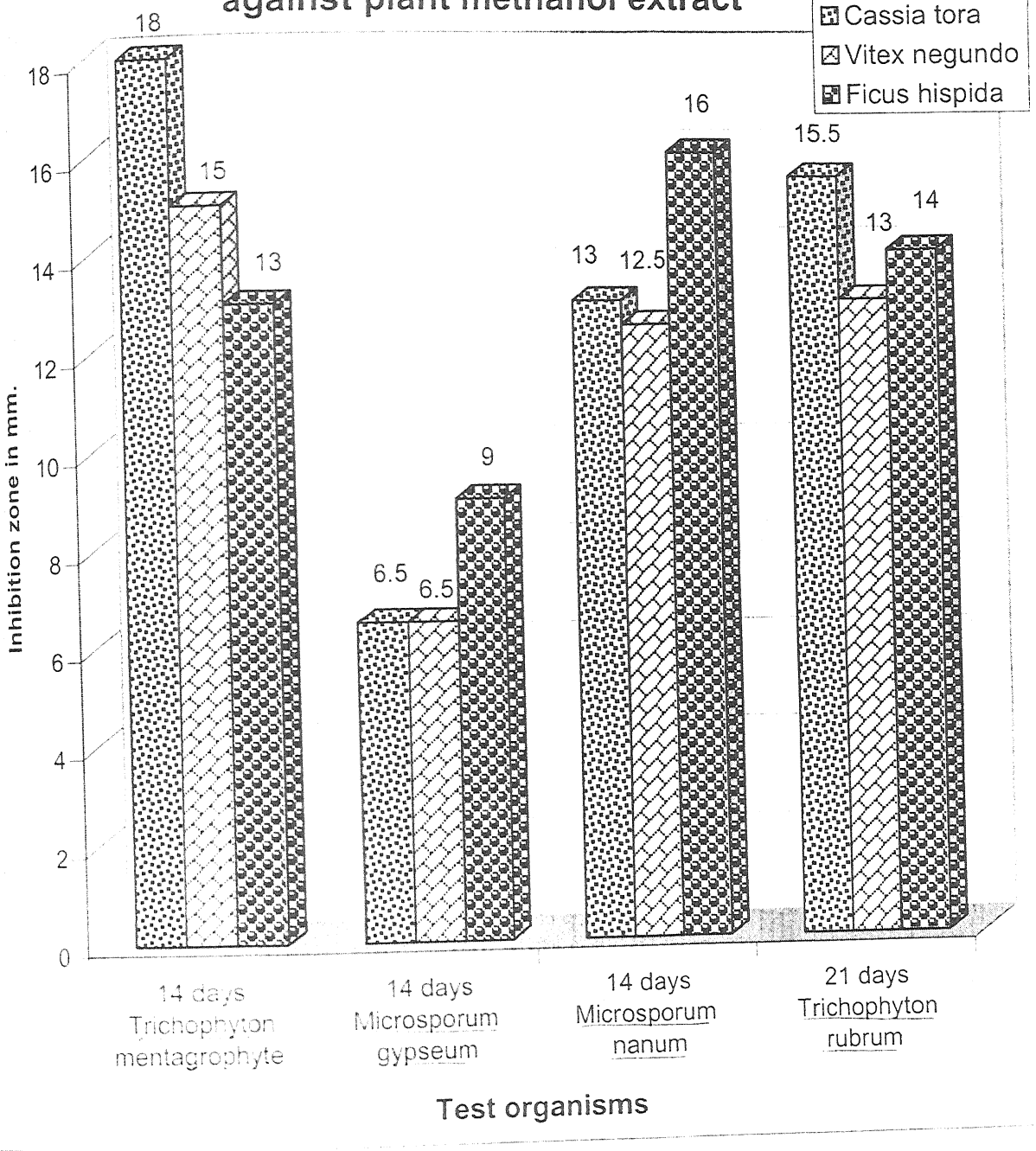
TABLE – VIII

Inhibitory effect of test organisms against plantsmethanol extract

(Inhibition zone in mm.)

Organisms	Observation Days	Cassia tora	Vitex negundo	Ficus hispida
Trichophyton mentagrophyte	14	18	15	13
Microsporum gypseum	14	6.5	6.5	9
Microsporum nanum	14	13	12.5	16
Trichophyton rubrum	21	15.5	13	14

FIG. 8 Inhibitory effect (mm) of test organisms against plant methanol extract



plants. solvent extracts however gave almost similar result, as there was not much difference in their inhibitory zones.

From the overall result it can be observed that both the two species of Trichophyton that is Trichophyton mentagrophyte and Trichophyton rubrum were best inhibited by Cassia tora extract. While the two species of Microsporum that is Microsporum gypseum and Microsporum nanum were best inhibited by Ficus hispida. Vitex negundo was generally found intermediate between the two plant extracts acetone extract was better inhibitory as compared to the methanol extract. These data can be observed from the table VIIth and VIIIth.

Antifungal study of oils extracted from active plants :

This study was carried with the essential oil extracted from active plant material in perkins apparatus. The extracted oil was diluted with acetone so that they could freely diffuse in agar medium. These samples were placed in glass cylinders fixed on seeded Sabouraud's agar medium. The inhibitory zone obtained after 14 days and 21 days have been mentioned in the table IX. The results obtained clearly show that these oils were more effective as compare to the solvent extracts.

Trichophyton mentagrophyte was best inhibited by Cassia tora oil followed the oils of Vitex nigundo and Ficus hispida. Cassia tora oil produced inhibition zone of 21 mm. while Vitex negundo

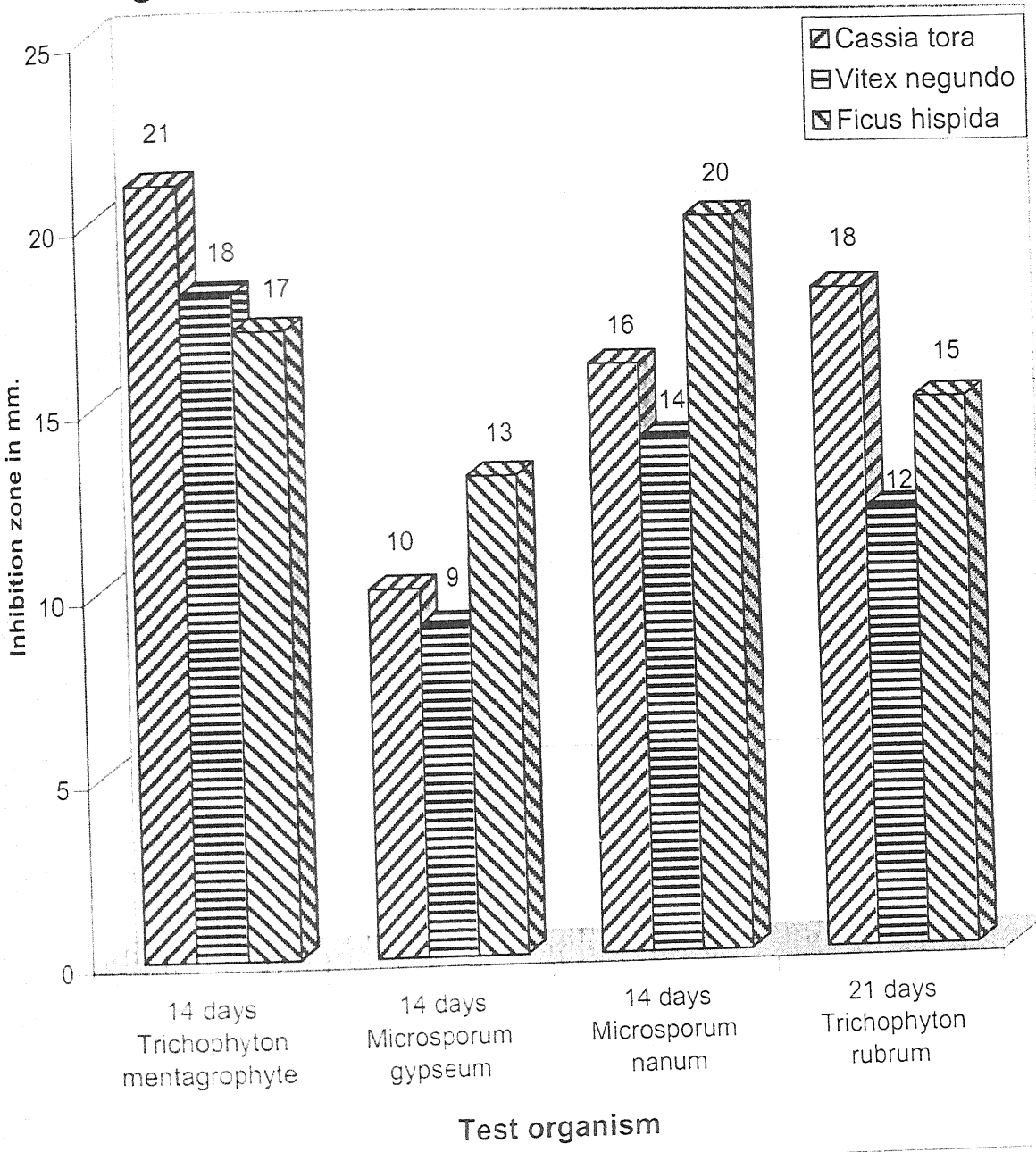
TABLE – IX

Inhibitory effect of test organisms against oils extracted from active plants

(Inhibition zone in mm.)

Organisms	Observation Days	Cassia tora	Vitex negundo	Ficus hispida
Trichophyton mentagrophyte	14	21	18	17
Microsporum gypseum	14	10	9	13
Microsporum nanum	14	16	14	20
Trichophyton rubrum	21	18	12	15

FIG. 9 Inhibitory effect (mm) of test organisms against oil extracted from active plant parts



oil produced 18 mm. inhibitory zone and that of Ficus hispida produced 17 mm. inhibitory zone.

Microsporum gypseum was inhibited best by Ficus hispida oil followed by Cassia tora and Vitex negundo oils. The inhibitory zones respectively were of 13 mm, 10 mm and 9 mm.

Microsporum nanum was best inhibited by Ficus hispida oil with inhibition zone of 20 mm followed by Cassia tora oil with 16 mm inhibition zone and Vitex negundo oil with 14 mm. inhibition zone.

Trichophyton rubrum was slow growing therefore it's inhibition zone was noted after 21 days of incubation. Best inhibitory effect was observed in Cassia tora oil followed by the oils of Ficus hispida and Vitex negundo here inhibitory zones of 18, 15, 12 mm. respectively were noted.

Again as observed in the previous experiment Cassia tora oils gave better inhibitory effect against the two species of Trichyphyton while Ficus hispida oil gave better results against two species of Microsporum.

Antifungal activity of composite samples of selected plant materials :

This experiment was performed with a view to get a composite sample which could inhibit all the four fungal organisms so that

it could be used for controlling infections caused by any of these fungus.

In sample one where all the three acetone solvents extracts were mixed in equal proportions. It was found that the inhibitory effect varied from one fungal organism to another. Best results were obtained against Trichophyton mentagrophyte where inhibition zone of 12 mm. was observed. Against Microsporum nanum and Trichophyton rubrum inhibitory zone of 10 mm were observed while against Microsporum gypseum 8 mm. inhibitory zone was observed.

In sample two where composite samples were made with acetone extracts of Cassia tora, Vitex negundo and Ficus hispida in a ratio of 1 :.5 :.5 gave better result for Trichophyton mentagrophyte followed by Trichophyton rubrum, Microsporum nanum and Microsporum gypseum.

In sample three where Cassia tora, Vitex negundo and Ficus hispida acetone extract were mixed in ratio of.5 : 1:.5 the results were better for Trichophyton mentagrophyte followed by Trichophyton rubrum, Microsporum nanum and Microsporum gypseum.

In the fourth sample where the three solvent extract were mixed in ration of.5 :.5 : 1 the results were better for Microsporum nanum followed by Microsporum gypseum, Trichophyton mentagrophyte & Trichophyton rubrum. These data's can be

TABLE – X

Inhibitory effect of test organisms against composite sample of active plants solvent extracts

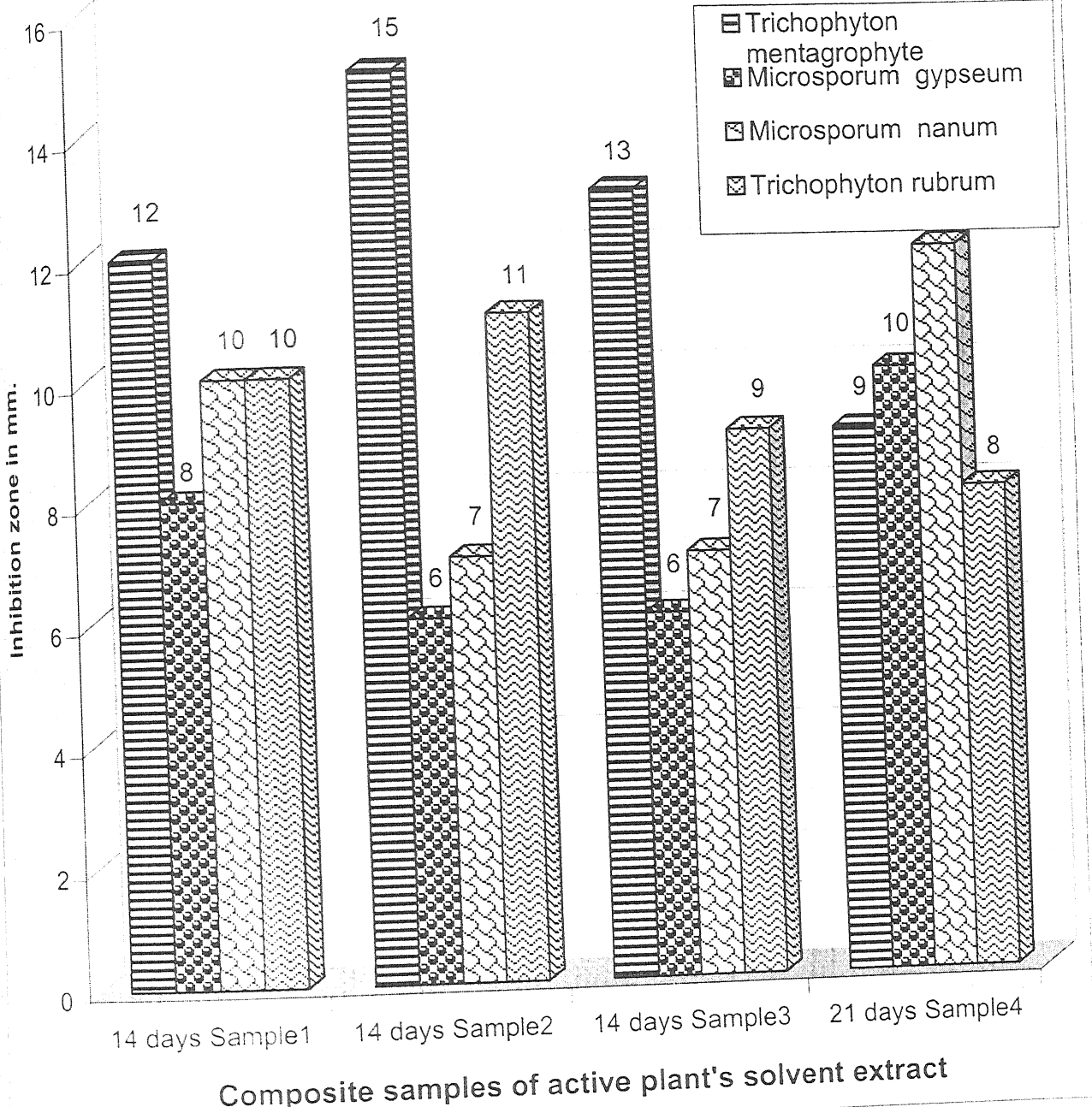
(Inhibition zone in mm.)

Organisms	Observation Days	Sample 1	Sample 2	Sample 3	Sample 4
Trichophyton mentagrophyte	14	12	15	13	9
Microsporum gypseum	14	8	6	6	10
Microsporum nanum	14	10	7	7	12
Trichophyton rubrum	21	10	11	9	8

Differenet proportion of plant solvent extract in composite samples

Composite sample	Cassia tora	Vitex negundo	Ficus hispida
1	1 ml.	1 ml.	1 ml.
2	1 ml.	0.5 ml.	0.5 ml.
3	0.5 ml.	1 ml.	0.5 ml.
4	0.5 ml.	0.5 ml.	1 ml.

FIG. 10 Inhibitory effect (mm) of test organisms against composite sample



observed from the table Xth. From these results it can be observed that the inhibitory zone were less as compared to inhibitory zones when solvent extract of only one plant was used. It appears that in combination their results were not as good. No explanation can be given for this reduction in their antifungal activity.

In general, it can be observed that when the composite sample was dominated with Cassia tora solvent extract better results were observed for Trichophyton mentagrophyte and Trichophyton rubrum and when Ficus hispida solvent extract dominated the composite samples, better results were observed for Microsporum gypseum & Microsporum nanum. It appears that in such samples only two solvent extract should have been used that is of Cassia tora & Ficus hispida, which might have given better result.

Antifungal activity of solvent extract on spore germination :

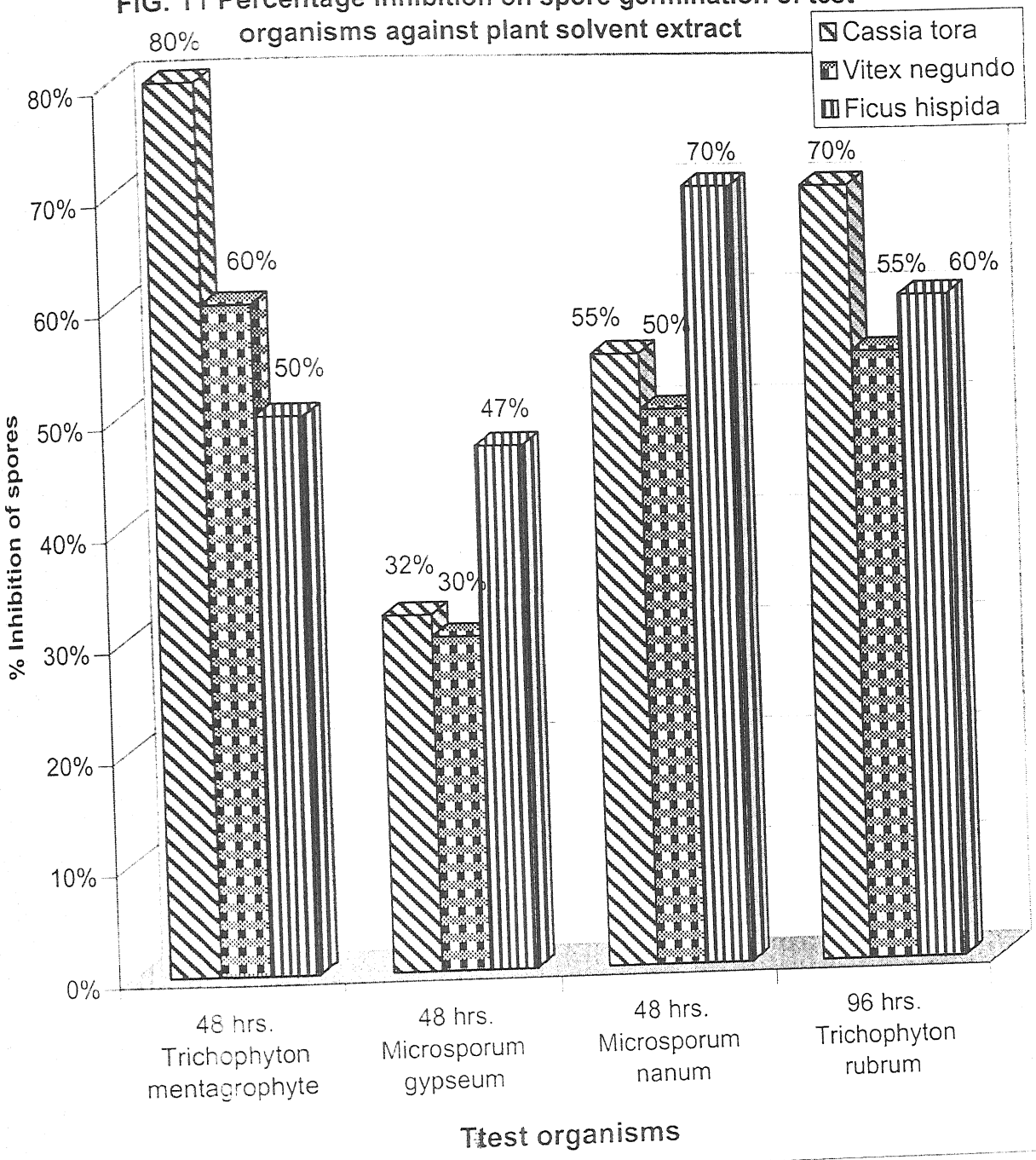
This experiment was conducted to find out the inhibitory effect of solvent extract on germination of spores, to find out weather the plant extract inhibit hyphal growth or germination of spores. The percentage inhibition on germination of spores has been obtained after 48 and 96 hours, the data obtained has been mentioned in table XI. From the data, obtained it can be observed that Trichophyton mentagrophytes spores are 80% inhibited when treated with Cassia tora extract; 60% in Vitex negundo extract and 50% in Ficus hispida extract. Microsporum

TABLE – XI

Percentage inhibition on spore germination of test organisms against plant solvent extract

Organisms	Observation Days	Cassia tora	Vitex negundo	Ficus hispida
Trichophyton mentagrophyte	14	80%	60%	50%
Microsporum gypseum	14	32%	30%	47%
Microsporum nanum	14	55%	50%	70%
Trichophyton rubrum	21	70%	55%	60%

FIG. 11 Percentage inhibition on spore germination of test organisms against plant solvent extract



gypseum had less inhibitory effect as 32% inhibition was found with Cassia tora, 30% with Vitex negundo and 47% with Ficus hispida extracts. Microsporum nanum had maximum inhibitory effect with Ficus hispida extract where 70% inhibition was found. Cassia tora extract was next with 55% inhibition and Vitex negundo extract was third with 50% inhibition.

Trichophyton rubrum spores were 70% inhibited with Cassia tora, 60% with Ficus hispida extract and 55% with Vitex negundo extract. From the data obtained it appears that these extracts inhibited both the spore germination as well as fungal hypha development. Again Cassia tora was more active on Trichophyton species and Ficus hispida on Microsporum species.

Minimum inhibitory concentraiton of oils :

This concentration is one, which is that minimum concentration of the oil, which could inhibit the growth of organism and could be used against the test pathogen for controlling the growth without causing any overdosing when used for in-vivo test. For this five different concentration of the oil were prepared. These were 5000, 2500, 1250, 625 and 312ppm. of each oil. These were prepared by dissolving the requisite amount in acetone. The inhibitory zone obtained has been mentioned in the table XII after 14 days of incubation. From the table it can be observed that the inhibitory zone gradually decreased with the rise in the concentration of oils. At 625ppm concentration of the oil no inhibition zone was observed for any of the test organism. The

TABLE – XII

Inhibitory effect of different oil dilutions against test organism

(Inhibition zone in mm.)

Plant	P.P.M oncentra -tion	T. ment-agrophyte	T. rubrum	M. gypseum	M. nanum
Cassia tora	5000	18	15	7	13
	2500	10	8	4	7
	1250	3	2	1	2
	625	1	-	-	-
	312	-	-	-	-
Vitex negundo	5000	15	9	6	12
	2500	8	5	4	7
	1250	3	2	1	2
	625	1	-	-	-
	312	-	-	-	-
Ficus hispida	5000	14	12	10	17
	2500	6	5	4	8
	1250	3	2	1	4
	625	1	-	-	1
	312	-	-	-	-

inhibition zone started from 1250 ppm concentration of all the three oils therefore this concentration was regarded as minimum inhibitory concentration of the oils.

Fungistatic or Fungicidal nature of Oils :

This experiment was conducted to study the fungicidal or the fungistatic concentrations of oils against the test pathogens. The concentrations used were 5000, 2500, 1250, 625, 312 ppm concentrations. The data observed have been recorded in the table XIII, XIV and XV.

From the observation recorded in Table XIII it can be observed that 5000 and 2500 ppm concentration of Cassia tora oil was fungicidal as no fungal growth could be observed either on the treated agar or on the fresh agar medium. At 1250 ppm concentration the oil showed fungicidal activity against Trichophyton mentagrophyte while against Trichophyton rubrum. Microsporum gypseum and Microsporum nanum this concentration found to be fungistatic as no growth was observed on the treated agar medium while fungal growth was observed when the disk were revived on fresh Sabouraud's agar medium.

Vitex negundo oil was fungicidal at 2500 ppm and above concentration but at lower concentration at 1250 ppm it gave fungicidal activity against Trichophyton mentagrophyte and fungistatic activity against rest of the three pathogens. The data obtained has been, mentioned in table XIV.

TABLE – XIII

Fungistatic or fungicidal activity of Cassia tora oil against test organisms

P.P.M Concentration	Trichophyton mentagrophyte		Trichophyton rubrum		Microsporum gypseum		Microsporum nanum	
	T	R	T	R	T	R	T	R
5000	-	-	-	-	-	-	-	-
2500	-	-	-	-	-	-	-	-
1250	-	-	-	+	-	+	-	+
625	+	+	+	+	+	+	+	+
312	+	+	+	+	+	+	+	+

T Test organism

R Re inoculated

\+ Presence of mycelial growth

\- Absence of mycelial growth

TABLE – XIV

Fungistatic or fungicidal activity of Vitex negundo oil against test organisms

P.P.M Concentration	Trichophyton mentagrophyte		Trichophyton rubrum		Microsporum gypseum		Microsporum nanum	
	T	R	T	R	T	R	T	R
5000	-	-	-	-	-	-	-	-
2500	-	-	-	-	-	-	-	-
1250	-	-	-	+	-	+	-	+
625	-	-	-	+	+	+	+	+
312	+	-	-	+	+	+	+	+

T Test organism

R Re inoculated

\+ Presence of mycelial growth

\- Absence of mycelial growth

TABLE – XV

Fungistatic or fungicidal activity of Ficus hispida oil against test organisms

P.P.M Concentration	Trichophy-ton ment-agrophyte		Trichoph-yton rubrum		Microspor-um Gypseum		Microspo-rum nanum	
	T	R	T	R	T	R	T	R
5000	-	-	-	-	-	-	-	-
2500	-	-	-	-	-	-	-	-
1250	-	-	-	+	-	+	-	-
625	-	-	+	+	+	+	-	+
312	-	-	+	+	+	+	+	+

T Test organism

R Re inoculated

\+ Presence of mycelial growth

\- Absence of mycelial growth

Ficus hispida oil was found to be fungicidal at 2500 ppm and above concentration against all the four test organisms. As no fungus could grow neither treated on agar petridish nor they could revive on fresh petridish.

At 1250 ppm concentration Ficus hispida oil was fungicidal against Trichophyton mentagrophyte and Microsporum nanum but this concentration of the oil was fungistatic against Trichophyton rubrum and Microsporum gypseum. At lower concentration the growth of the test organism were observed and therefore these concentrations could be said to be ineffective. This experiment shows that in all the oil used 2500 ppm concentrations and above could be used as fungicidal concentration. Controls were run side by side in which only petroleum jelly were used without adding oil. The data recorded have been mentioned in table XV.

Sensitivity test of oils obtained from active plant material on human skin :

This experiment was performed to study the sensitivity of different oils if applied on human skin. The observations recorded have been given in the table XVI after 10 days of application. The observation recorded clearly shows that the oils did not produce any allergic response when applied to different persons. With Cassia tora oil out of 25 persons only one complained of mild irritation and one had soothing effect. The rest 23 had no effect. In Vitex negundo oil out of 25 persons

TABLE – XVI

Sensitivity test on human skin of different oils obtained from active plants material

Response	Control	Cassia tora	Vitex negundo	Ficus hispida
No effect	25	23	22	20
Soothing effect		1	2	3
Irritation (mild)		1	1	2
Allergic response		-	-	-

tested one showed irritation; two soothing effect and rest 22 had no effect.

In Ficus hispida oil out of 25 presons tested 2 complained for mild irritation and 3 had soothing effect the rest 20 had no effect. None of the person suffered from allergic responses from any of the oil tested.

Efficiency of the oil for the cure of infection :

Before the application of ointment confirmation for the establishment of the infection was done on patients. For this study both direct examination and culture positive test were made. For direct examination the sample were collected from infected area and placed on clean slide with one drop of aqueous 10% KOH solution to dissolve the keratin and to separate the mycelium & conidia. The presence of mycelium and/or conidia of the test pathogen showed the establishment of the disease by the pathogen. From these patients confirmation was also made by culture test. For this study subouraud's agar medium was prepared with cycloheximide and chloramphenicol. This medium was poured into pre sterilized petri plates. After solidification the medium was kept ready for cultural test. The cut pieces of the hairs and skin scraping were aseptically transferred onto the medium and incubated 28^0C. The culture obtained was examined under the microscope for confirmation of the disease development by test fungus.

After confirmation, the in-vivo study were made by the ointment of the oils prepared by mixing 1 ml. of the oil into 100 gm of petroleum jelly, this ointment was used twice in a day (one in the morning and other in evening). The examination of treatment was made by placing the hairs/skin scraping collected from the border of infection side on alternate days by the method already described above. The results were recorded in terms of percent culture recovery following Waheb et. al., 1982 by the formula given below.

$$\text{Recovery Percentage} = \frac{\text{Total no. of sites for positive tests}}{\text{Total no. of sites examined}} \times 100$$

The data obtained are being mentioned in the table XVII, XVIII and XIX. It should be made clear that in-vivo test could be made only against three types of infections caused by Trichophyton mentagrophyte, Trichophyton rubrum and Microsoproum gypseum as during in-vivo test, no patient suffering from Microsporum nanum could be found. In vivo test were performed only with two oils Cassia tora and Ficus hispida as these were giving better results against the pathogen as compared to Vitex negundo oil as found in the previous experiments.

The results obtained in the table XVII, XVIII and XIX indicate that Trichophyton mentagrophyte infection could be cured completely after 21 days treatment of Cassia tora oil and 23 days treatment of Ficus hispida oil.

TABLE – XVII

Efficiency of oil ointment against infection caused by

Trichophyton mentagrophyte

Treatment days	Present culture recovery (%)		
	Control	Cassia tora	Ficus hispida
3	100%	100%	100%
5	100%	100%	100%
7	100%	100%	100%
9	100%	100%	100%
11	100%	100%	100%
13	100%	100%	100%
15	100%	75%	100%
17	100%	30%	70%
19	100%	10%	35%
21	100%	0%	10%
23	100%	0%	0%

TABLE – XVIII

Efficiency of oil ointment against infection caused by

Trichophyton rubrum

Treatment days	Present culture recovery (%)		
	Control	Cassia tora	Ficus hispida
3	100%	100%	100%
5	100%	100%	100%
7	100%	100%	100%
9	100%	100%	100%
11	100%	100%	100%
13	100%	100%	100%
15	100%	100%	100%
17	100%	85%	80%
19	100%	40%	50%
21	100%	10%	20%
23	100%	0%	10%

TABLE – XIX

Efficiency of oil ointment against infection caused by *Microsporum gypseum*

Treatment days	Percentage culture recovery		
	Control	Cassia tora	Ficus hispida
3	100%	100%	100%
5	100%	100%	100%
7	100%	100%	100%
9	100%	100%	100%
11	100%	100%	90%
13	100%	100%	65%
15	100%	80%	30%
17	100%	45%	10%
19	100%	20%	0%
21	100%	10%	0%
23	100%	0%	0%

Trichophyton rubrum infection could be cured with 23 days treatment of Cassia tora oil and more than 23 days treatment with Ficus hispida oil. Microsporum gypseum in infection could be cured, 23 days treatment of Cassia tora oil and 21 days treatment of Ficus hispida oil. It can be observed that Trichophyton could be better cured with Cassia tora oil while Microsporum could be better cured with Ficus hispida oil.

Effect of Plant material on Bio-chemical parameters of blood in Albino rat :

This was done with the view to study the effect of plants oil when they come in contact with the blood. Since any physiological or other variation due to stress and strain or absorption of substance brings change in the physiology and biochemical distribution of blood which affect the other systems of the body. It gives a better under standing of the metabolic study and is also useful in determining the deleterious effect of the substance, which comes in contact with the blood. It helps in designing the future strategy in drug induced management in higher mammals. Since rats are used in most of the biological studies, in present study also these were employed to study the effect of oils on the following parameters.

Behaviour changes :

The behaviour changes were observed after every seven days during regular application of oil in petroleum jelly. It was found

that experimental animals behaved normally without any saliva from the mouth, legs & body tremors, immovability and prostration etc. up to 21 days of application.

Food consumption :

The average food consumed by the rats treated with Cassia tora oil ointment on the first day was 16 gm. while in the treatment rat of Cassia tora ointment it was 14 gm. as such there was a decrease of 2 gm. while in those rats treated with Ficus hispida ointment in control rats there was an average of 16 gm. as compare to 13 gm. in the treated rat, showing a decrease of 3 gm. After 7 days treatment of Ficus hispida ointment the treated rats showed a mean value of 12 gm/day. While in the control rats it was 15gm/day. After 14 days in control rats the food consumption ranged from 08 gm/day to 20 gm/day with an average of 14 gm/day while in treated rat the value ranged from 8 gm/day to 20 gm/day averaging 13gm/day. After 21 days food consumption by rats in the control rats had an average of 16 gm/day and in the treated rats there was an average of 13th gm/day. (Table no. XX & XXI). As will be evident from the table the consumption was slightly higher, in the control rat as compare to the treated rat. The difference is not much and therefore is quite insignificant.

Body Weight :

The body weight of rats in untreated rats with Cassia tora ointment ranged from 204 to 211 gm with an average of 205 gm.

TABLE – XX

Food consumption in Cassia tora oil ointment treated and controlled Rattus norvegicus

S.N.	Observation Day	Food consumed by three treated rats (gm)			Mean	Food consumed by three controled rats (gm)			Mean
		1	2	3		1	2	3	
1	1	13	15	14	14	15	16	17	16
2	7	12	18	15	15	14	17	19	17
3	14	14	15	19	16	13	15	14	14
4	21	15	15	14	15	19	21	15	18

TABLE – XXI

Food consumption in Ficus hispida oil ointment treated and controlled Rattus norvegicus

S.N.	Observation Day	Food consumed by three treated rats (gm)			Mean	Food consumed by three controlled rats (gm)			Mean
		1	2	3		1	2	3	
1	1	10	11	18	13	14	12	22	16
2	7	9	12	15	12	11	14	20	15
3	14	8	10	20	13	09	14	20	14
14	21	8	13	19	13	11	18	19	16

While in the treated rats the body weight ranged from 200 gm to 236 gm with an average of 214 gm. This increase in the body weight was quite insignificant. In rats treated with Ficus hispida ointment, the body weight ranged from 205 to 225 gm with an average of 212 gm. In the control rat the body weight range from 200 to 220 gm. averaging 210 gm on the first day. After seven days with Cassia tora treatment control rats averaged was 225 gm while in the treated rats average was 220 gm. With Ficus hispida ointment control rats had an average of 220 gm. while the treated rats had an average of 224 gm. After 14 days treatment in rats having Cassia tora treatment control rats had an average of 206 gm. While, in the treated rate it was 208 gm. in rats having treatment with Ficus hispida ointment control rate had an average weight of 225 gm while the treated rats had an average of 220 gm. In rats with Cassia tora after 21 days the control rats had 208 gm average body eight while that of treated rats had an average of 219 gm. In rats with Ficus hispida treatment the average body weight was 236 gm while in untreated control rats had an average of 231 gm. The above result have been shown in the Table XXII & XXIII.

From these data it can be observed that the body weight in general did not vary much in control and treated rats.

Total erythrocyte count :

The total erythrocyte count in rats treated with Cassia tora and Ficus hispida ointment has been noted after 1,7,14 and 21 days treatment.The result obtained have been shown in the table

TABLE – XXII

Body weight under Cassia tora oil ointment treated and controlled Rattus norvegicus

S.N	Observation Day	Weight of three Controlled rats (gm)			Mean	Weight of three Treated rats (gm)			Mean
		1	2	3		1	2	3	
1	1	204	200	211	205	200	206	236	214
2	7	210	238	227	225	216	214	230	220
3	14	206	199	213	206	200	213	211	208
4	21	205	204	215	208	210	217	230	219

TABLE – XXIII

Body weight under Ficus hispida oil ointment treated and controlled Rattus norvegicus

S.N.	Observation Day	Weight of three Controlled rats (gm)			Mean	Weight of three Treated rats (gm)			Mean
		1	2	3		1	2	3	
1	1	200	210	220	210	205	206	225	212
2	7	211	219	230	220	214	238	220	224
3	14	212	228	235	225	220	218	222	230
4	21	219	244	230	231	225	256	227	236

XXIV. The control rats without Cassia tora oil treatment average value obtained were the same as obtained in the control rat without Ficus hispida oil treatment. Both had an average of 3.9 mill/mm^3. After the treatment on first day in Cassia tora oil ointment average value obtained was 3.4 Mill/mm^3 while after the treatment of Ficus hispida ointment, the value was 4.2 mill/mm^3. After seven days treatment the average value in Cassia tora ointment was 3.2 mill/mm^3, while that of Ficus hispida ointment was 4 mill/mm^3. After 14 days teatment in Cassia tora ointment Erythrocyte count had an average of 3.4 mill/mm^3 and in Ficus hispida ointment. The value was 3.5 mill/mm^3. After 21 days treatment with Cassia tora ointment the value ranged from 3.3 mill/mm^3 to 4.2 mill/mm^3 with an average of 3.7 mill/mm^3. In Ficus hispida ointment the value ranged from 3.2 mill/mm^3 to 4.2 mill/mm^3 with an average of 3.8 mill/mm^3. The over all study showed that neither Cassia tora ointment nor Ficus hispida ointment had any adverse effect on the total erythorocyte count.

Total leucoycyte count :

The values obtained of total leucocytes count in rats after treatment with Cassia tora ointment and Ficus hispida ointment has been mentioned in the table XXV. In the control rats without both the treatment the average values were 11, Th/mm^3 and 10 Th/mm^3 respectively. After 1 day treatment it increased to 14 Th/mm^3 in the rats having treatment of Cassia tora ointment, while in the treatment of Ficus hispida ointment the mean value was 15 Th/mm^3. After 7 days treatment the mean value obtained

TABLE – XXIV

Total erythrocyte count (mill/mm^3) under Cassia tora/Ficus hispida oil ointment treated and controlled conditions of Rattus norvegicus

S.N.	Observation Day	Cassia tora oil treatment			Mean	Ficus hispida oil treatment			Mean
		1	2	3		1	2	3	
1.	1	3.2	3.3	3.7	3.4	3.6	4.5	4.5	4.2
2.	7	2.8	2.9	3.9	3.2	0.4	3.0	5.0	4.0
3.	14	2.3	4.4	3.5	3.4	3.5	3.3	3.8	3.5
4.	21	3.3	3.6	4.2	3.7	3.2	4.2	4.0	3.8
Control		3.4	3.5	4.8	3.9	2.9	4.0	4.8	3.9

TABLE – XXV

Total leucocyte count (Th/mm^3) under Cassia tora/Ficus hispida oil ointment treated and controlled conditions of Rattus norvegicus

S.N.	Observation Day	Cassia tora oil treatment			Mean	Ficus hispida oil treatment			Mean
		1	2	3		1	2	3	
1.	1	13	12	17	14	15	16	14	15
2.	7	11	13	15	13	12	15	15	14
3.	14	12	10	14	12	10	13	13	12
4.	21	14	12.4	12	12.8	11	12.2	14	12.4
Control		8	12	13	11	8	10	12	10

in Cassia tora ointment was 13 Th/mm^3, while in Ficus hispida treatment it was 14 Th./mm^3. After 14 days treatment the leucocytes count in the rats having treatment of Cassia tora ointment the average value was 12 Th/mm^3 the same average value was obtained in Ficus hispida oil treatment. After 21 days treatment with Cassia tora ointment the leucocyte count ranged from 12 Th/mm^3 to 14 Th/mm^3 with an average of 12.8 Th/mm^3. When treatment was made with Ficus hispida ointment the value ranged from 11 Th/mm^3 to 14 Th/mm^3 with an average of 12.4 Th/mm^3. The values obtained in the table XXV clearly indicate that the leucocyte count has slightly increased from the control rat which might be due to the cause of injury to the rats while applying the ointments.

Hemoglobin Content :

The hemoglobin concentration in the control rats ranged from 12.5 to 14.1 gm/100 ml with an average of 13.2 gm/100ml in case of without Cassia tora and in case of without Ficus hispida oil treatment it ranged from 12.4 to 14.1 gm/100 ml with an average of 13.3 gm/100ml as shown in the table XXVI. On the first day of treatment the average value in Cassia tora ointment was 12.6 gm/100 ml and in Ficus hispida ointment the average value was 13.5 gm/100 ml. On the seventh day of treatment in case of Cassia tora ointment the average value was 12.5 gm/100 ml., while that of Ficus hispida ointment the average value was 14.2 gm/100 ml. After 14 days of treatment the average value in Cassia tora ointment was 12.2 gm/100 ml. while that of Ficus

TABLE – XXVI

Hemoglobin count (gm./100ml) under Cassia tora/Ficus hispida oil ointment treated and controlled conditions of Rattus norvegicus

S.N.	Observation Day	Cassia tora oil treatment			Mean	Ficus hispida oil treatment			Mean
		1	2	3		1	2	3	
1.	1	12.3	12.5	13.0	12.6	13.0	13.3	14.2	13.5
2.	7	12.2	12.2	13.1	12.5	14.1	14.3	14.2	14.2
3.	14	11.8	11.3	13.5	12.2	11.8	13.3	12.4	12.5
4.	21	13.4	13.5	13.2	13.4	12.4	12.8	13.2	12.8
Control		12.5	13.0	14.1	13.2	12.4	14.1	13.5	13.3

hispida it was 12.5 gm/100 ml. After 21 days of treatment the average value in case of Cassia tora was 13.4 gm/100 ml. while that of Ficus hispida was 12.8 gm/100 ml. The entire data in the table show that the variation of hemoglobin was not much and this show that both the ointment had not affected the hemoglobin contents of rats. It maintained almost the same level.

Blood glucose :

The label of blood glucose as observed on rats after treatment with Cassia tora & Ficus hispida ointment. Data obtained has been entered in the table XXVII. A perusual of the table shows the blood glucose label in the control rats ranged from 140 to 152 mg/100 ml with an average of 145 mg/100 ml. in case of rats without Cassia tora treatment. In rats of without Ficus hispida ointment the value ranged from 141 mg/100 ml. to 150ml./100 ml. with an average of 147 ml./100 ml. On the first day of treatment with Cassia tora ointment the average value was 140 mg/100 ml. while in the treatment of Ficus hispida ointment the average value was of 144 mg/100 ml. After seven days treatment value recorded in the treatment of Cassia tora ointment was 142 mg/100 ml. while in that of Ficus hispida ointment the mean value was of 146 mg/100 ml. After 14 days treatment the average value in the treatment of Cassia tora ointment was 141 mg/100 ml. while that of Ficus hispida ointment mean value was 145 mg/100 m. After 21 days treatment with Cassia tora ointment the average value was 148 mg/100 ml. while in case of Ficus hispida ointment the average value was also 148 mg/100 ml. This shows

TABLE – XXVII

Blood glucose level (mg./100ml) under Cassia tora/Ficus hispida oil ointment treated and controlled conditions of Rattus norvegicus

S.N.	Observation Day	Cassia tora oil treatment			Mean	Ficus hispida oil treatment			Mean
		1	2	3		1	2	3	
1.	1	138	136	146	140	140	150	142	144
2.	7	139	143	144	142	141	154	143	146
3.	14	140	137	146	141	142	146	147	145
4.	21	142	153	149	148	144	158	142	148
Control		140	143	152	145	141	150	150	147

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that even the blood glucose label was not alter by the treatment of either Cassia tora ointment or Ficus hispida ointment. This show that both the ointment don't have any effect on the blood glucose label of rats.

Serum Cholesterol :

The values obtained of serum cholesterol label from the treated rat have been mentioned in the table XXVIII. The values shown in the table indicate that in the control rats without Cassia tora treatment the value ranged from 227.1 mg/100 ml. to 279.8 mg/100 ml with an average value of 253.4 mg/100 ml. Similarly in control rat without Ficus hispida treatment the value ranged from 238.2 mg/100 ml. to 271.6 mg/100 ml. with a mean value 249.2 mg/100 ml. On the first day of treatment with Cassia tora ointment the mean value obtained was 240.3 mg/100 ml. while that of Ficus hispida ointment the mean value was 244.2 mg/100 ml. After seven days treatment with Cassia tora ointment the mean value was 244.4 mg/100 ml. while with that of Ficus hispida ointment was 241.2 mg/100 ml. After 14 days treatment with Cassia tora ointment the mean value obtained was 241.2 mg/100 ml while that of Ficus hispida ointment was 250.2 mg/100 ml. After 21 days treatment the mean value in the treatment of Cassia tora ointment was 243 mg/100 ml. while that of Ficus hispida ointment was 248.2 mg/100 ml. These values show that even the serum cholesterol label have not been much altered with the treatment of either of the ointment. It maintains almost the same label as found in the control rats.

TABLE – XXVIII

Serum cholesterol level (mg./100ml) under Cassia tora/Ficus hispida oil ointment treated and controlled conditions of Rattus norvegicus

S.N.	Observation Day	Cassia tora oil treatment			Mean	Ficus hispida oil treatment			Mean
		1	2	3		1	2	3	
1.	1	227.6	261.1	232.2	240.3	229.4	272.9	230.3	244.2
2.	7	229.7	269.5	234.2	244.4	230.2	259.4	234.2	241.2
3.	14	232.8	251.6	239.2	241.2	239.2	275.2	236.2	250.2
4.	21	242.2	254.5	232.3	243.0	229.2	274.1	241.3	248.2
Control		227.1	279.8	253.3	253.4	238.2	271.6	238.0	249.2

SECTION 5

SUMMARY AND DISCUSSION

CHAPTER – 5

Summary & Discussion

The present investigation was undertaken to "Studies on Antifungal and Biochemical parameters of plant material with special reference to Dermatophytosis". During the present investigation antifungal potency of higher plants against Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum and Microsporum nanum were studied both in-vitro and in-vivo in order to develop potent indigineous herbal therapeutant so that the various side effect found in synthetic drug could be over come. The findings of the present study are being discussed in the light of previous investigations.

Isolations of dermatophytes :

The isolation of dermatophytes were made from the O.P.D. section of dermatology department of M.L.B. Medical College, Jhansi under the supervision of Dr. Dinesh Govil. The isolates were brought to the lab for isolation & identification of the fungus on Sabouraud's agar media. Out of 84 samples obtained 31 samples were of Trichophyton rubrum, one of Microsporum nanum, 40 of Trichophyton mentagrophyte, two of Microsporum gypseum and the rest samples were negative. This show that most of the patients were suffering from the infection of Trichophyton mentagrophyte followed by Trichophyton rubrum and then Microsporum gypseum while only one isolate was of

Microsporum nanum. Identification of isolates were confirmed on the basis of morphological and cultural characteristics consulting monographs and authentic cultures obtained from All India Institute of Medical Science, New Delhi.

Screening of plant for antifungal activity :

As described in the chapter "Introduction" large member of workers have screened higher plants against plant pathogens. However a systematic evaluation of higher plants against human pathogen remained comparatively neglected. In the present study 64 plants were screened which belong to twenty two families of Angiosperms. Out of these Cassia tora, Trachyspermum ammi, Ficus hispida and Vitex negundo showed maximum inhibitory effect against the four fungal organisms isolated from patients. The rest of the plant species exhibited varring degree of toxicity. Singh 1987 reported strong fungitoxic activity of the family Meliaceae.

Dixit & Tripathi 1975 found strong fungicidal toxicity in Caesalpinaceae. Giliver 1947; Dixit 1978; Singh et. al., 1986 found strong fungicidal activity in Umbelliferae. Kishor et. al., 1981, found Verbenaceae to be actively fungicidal. These results are in conformity with the results of the authors. Considering the findings of the previous workers that plant parts differ in fungitoxicity. Stwarts and Medrik 1968 found corn berry juice fungitoxic against dermatophyte. Ahmed et. al., 1977 found Juglans regia bark fungitoxic against Microsporum gypseum;

Tansey and Appleton 1975, found bulbs of Alluim sativum active against Microsporum gypseum and Trichophyton rubrum. Mukharya and Dahia 1977, found root of Plumbago species active against Microsporum gypseum. Chile et. al., 1981 found the entire plant of Vinca rosea active against Trichophyton rubrum in which the leaves showed maximum activity. Joshi and Bhatt 1983, found Chakramad B. durva active against Microsporum and Trichophyton species. Similarly many worker like Singh 1984; Rao & Rao 1985; Tripathi et. al., 1988; Gupta 1988; Mishra et. al., 1988; Tripathi et. al., 1990 etc. have found different parts or different plants, fungicidal against dermatophytes. The authors have found the positive activity of some of these plant but few of these showed significant activity and therefore were selected for further study. These results of the author are therefore in conformity to the result of the previous work. Various workers like Mishra have observed the fungitoxicity varied from genus to genus within a family as 1975; Tripathi 1980; Asthana 1984. Variations in fungitoxicity were also observed from species to species within the same genus. Out of the two species of genus Cassia only Cassia tora exhibited strong toxicity while the other showed poor toxicity. Thus plants containing fungitoxicity are scattered throughout the flowering plants and their activity is quite unrelated to their taxonomic position. Even within the some plant, some parts are more fungitoxic than the other part of the same plant. The leaves of the most of the plants were found to be more toxic than the other parts. In the present study, leaves of different plants were used during the preliminary screening later other parts were

studied. Since in the present study airdried parts were found to loose their antifungal activity thus the fresh part of the plants were used in the present study. This observation is inconformity to those of Gilliver 1947; Abdulla, 1959; Dubey 1981 and Asthana, 1984.

In the present study four plants out of 64 originally screened were selected to study the presence of fungitoxicity in different part of these plants. These plants were Cassia tora, Vitex negundo, Ficus hispida and Trachysperrmum ammi. It was observed that pods of Cassia tora, leaves of Vitex negundo and Ficus hispida, while seeds of Trachysperrmum ammi showed better fungitoxicity as compared to their other parts. From this experiment it was also observed that the above part of Cassia tora, Vitex negundo and Ficus hispida gave better results than Trachysperrmum ammi. Therefore three parts of the above plants were selected for further study.

Extracts of plant parts were employed for antifungal tests in the present study. For this water extracts and organic solvent extracts were used. Singh 1980; Dubey et. al., 1982; Pandey et. al., 1983; Asthana 1984; Chandra 1984; Mall 1987 and Gupta 1988 also used water extracts, while different organic solvents were employed by others like Tripathi, 1976, 1977; Chaturvedi, 1979; Dixit, 1980; Saxena 1980. In the present study aqueous extracts of selected parts of the plants were preferred for antifungal screening. Since water being the most polar solvent it facilitates the extractions of maximum constituents from the plant's part.

The water extract obtained from Cassia tora pods Vitex negundo and Ficus hispida leaves were mixed with sabouraud's agar media and their effect on the radial growth of the four test organism were studied. Radial growth of Trichophyton mentagrophyte, Microsporum Gypseum and Microsporum nanum were measured after every 24 hours while that of Trichophyton rubrum were measured after every 48 hours, because of the very slow growth of later fungus. Trichophyton mentagrophyte was found to be best inhibited by the immature pods extract of Cassia tora. Microsporum gypseum Microsporum nanum and Trichophyton rubrum were best inhibited by Ficus hispida leaves extract.

When the inhibitory effect was measured in terms of percentage inhibition a slightly different picture developed. Cassia tora water extract had highest percent inhibition against Trichophyton mentagrophyte followed by Trichophyton rubrum, Microsporum nanum and Microsporum gypseum. The inhibitory effect of Vitex negundo leaf extract was also found in the same sequence. On Ficus hispida leaf extract Microsporum nanum showed best percentage inhibition followed by Trichophyton mentagrophyte, Trichophyton rubrum and Microsporum gypseum respectively.

Antifungal study of Plants solvent extract :

Various workers have used organic solvents for extraction of fungitoxic constituents of higher plants. Doskotch et. al., 1975; Turner et. al., 1975; Dixit et. al., 1976; Tripathi et. al., 1978 used solvents. For this number of solvents are available for extraction of

different chemicals present in the plants. In the present study extracts from active plant parts were made in acetone and methanol solvents using Soxhlet apparatus. These were tested against the four test organism namely Trichophyton mentagrophyte, Trichophyton rubrum, Microsporum gypseum and Microsporum nanum respectively, using glass cylinder fixed on fungus seeded Sabouraud Agar media. The zone of inhibition obtained was noted.

From the results obtained it was found that acetone extract was better inhibitory as compared to methanol extract. The two species of Trichophyton that is Trichophyton mentagrophyte and Trichophyton rubrum were better inhibited by Cassia tora pod extract, while the two species of Microsporum that is Microsporum gypseum and Microsporum nanum were best inhibited by Ficus hispida leaf extract. Vitex negundo leaf extract was generally found to be moderately effective.

Antifungal study of oils extracted from active plant parts :

Extraction of fungitoxic constituent by hydrodistillation technique has also been adopted by various workers, such as Chaturvedi 1979; Grover and Rao 1979; Asthana et. al., 1982; Renu et. al., 1985. In the present study hydrodistillation was conducted by Perkin's apparatus, the extracted oils were used with acetone so that it could freely diffuse in agar medium. These oil samples were placed in glass cylinder fixed on test fungus seeded agar. Sabouraud's medium. The inhibitory zones developed were studied after 14 and 21 days. From this study it was found that Cassia tora oil produced best inhibitory effect on

Trichophyton mentagrophyte followed by that of Cassia tora and Vitex negundo, Microsporum nanum were best inhibited by Ficus hispida while Cassia tora and Vitex negundo oils were next in sequenc. Trichophyton rubrum was best inhibited by Cassia tora oil followed by Vitex negundo and Ficus hispida. Microsporum gypseum was best inhibited by Ficus hispida oil followed by that of Ficus hispida and Vitex negundo. These observations confirm above previous result obtained with solvent extract.

Previously many workers have used oils obtained from plants for their antinfungal activity. Asthana, 1984; Kishore 1985; Mall 1987 isolated oils from active plants while others like Sharma & Singh 1979 tested the commercial oil for their fungitoxicity. These workers have not paid much attention on the detail fungitoxic property such as its possible therapeutant use. In the present investigation oils were subjected to detail fungitoxic study. Such as minimum inhibitory concentration, fungicidal or fungislatic nature of oil, it's senstivity on human skin and efficiency of the oil for curing infections, there effect on biochemical parameters of blood in albino rats.

Antifungal activity of composite sample of selected plant material :

This experiment was conducted with a view to get a composite samples in which samples from all the three plants could be

mixed so that it could be used for controlling infections caused by any of these fungus.

In this experiment acetone solvent extract obtained from active plant part of three plants Cassia tora, Vitex negundo and Ficus hispida were mixed in different proportions. In all, four such composite sample were prepared. One in which all the three plant material were mixed in equal proportion and the other three in which one of the plant material was reduced to half the quantity of other two plant materials.

From this experiment it was observed that samples which had Cassia tora solvent extract in dominating position gave better result against Trichophyton mentagrophyte and Trichophyton rubrum when Ficus hispida extract dominated it gave better result against Microsporum gypseum and Microsporum nanum.

Antifungal activity of solvent extract on spore germination:

In this experiment inhibitory effect of solvent extract on spore germination of the four fungal organisms were studied. From this experiment it was found that Trichophyton mentagrophyte spores were 80% inhibited when treated with Cassia tora extract, 60% with Vitex negundo extract and 50% with Ficus hispida extract. Microsporum gypseum spores germinations were inhibited to 32%, 30% and 47% with extract of Cassia tora, Vitex negundo and Ficus hispida respectively. Microsporum nanum spores were 70%, 55% and 50% inhibited with Ficus

hispida, Cassia tora and Vitex negundo respectively. While Trichophyton rubrum spores were 70% inhibited with Cassia tora extract, 60% with Ficus hispide and 50% with Vitex negundo. From these data and those obtained on radial growth it appears that these plant extract inhibited both spore germination and fungal hyphae development.

Minimum inhibitory concentration :

This experiment is important specially when minimum doze of the fungitoxic substances is to be recommended when used for in-vivo test. Overdosing may result in wastage of the fungitoxic substance or may impart toxicity to the host.

For this five different concentrations of the oil were prepared in acetone, these were 5000, 2500, 1250, 625 and 312 ppm. of each oil. The inhibitory zones obtained measured after 14 days of incuabation, it was observed that inhibitory zone started from 1200 ppm concentration therefore this concentration was regarded as minimum inhibitory concentration of the oil.

Several persons have tried to determine the MIC of various essential oils. Pandey, et. al., 1983 has found Ageratum haustonianum oil to have 100 ppm MIC concentration against Microsporum gypseum. Singh et. al., 1986 has found 900 ppm concentration of Trachysperrmum ammi against Trichophyton mentagrophyte. In 1987 they found 1000 ppm concentration of Hyptis suaveolans to be MIC against the same fungus in 1983,

they found 1000 ppm concentration of Ocimum gratissium to be MIC against the same fungus.

The variation seems to be due to different sensitivity of particular oil against the test fungus it may also be due to different technique used during antifungal investigations. The MIC concentration obtained in the present in the present investigation is almost conformity to the above observations. The slight difference may be due to the nature of oil and sensitivity of the test organism towards the oil tested.

Fungistatic or fungicidal nature of oil :

The substances toxic to the fungi may inhibit the growth of fungi either temporarily of permanently. When fungi is temporarily inhibited the fungitoxic substances are regarded as fungistatic and when fungi is permanently inhibited the fungitoxic substances are fungicidal. Oils of Ageratum houstoniaum Pandey et. al., 1983; Ocimum Canum Dubey, 1981. Cympogon martini Singh et. al., 1980 showed fungistatic nature of the oils. While Hyptis suaveolans Pandey et. al., 1982. Chenopodium ambrosioides Kishore, 1985 exhibited fungicidal nature of the oil at MIC concentrations.

In the present study it was observed that all the three oils, obtained from Cassia tora, Vitex negundo and Ficus hispida were fungicidal at 2500 and 5000 ppm concentration against all the four fungal organisms. At 1250 ppm concentration all the

above three oils were fungicidal against Trichophyton mentagrophyte but fungistatic against Trichophyton rubrum Microsporum gypseum and Microsporum nanum. This shows that the oils showed fungistatic nature at their MIC concentration but at higher concentration they exhibited fungicidal nature. Thus their fungicidal or fungistatic nature is dose dependent.

Sensitivity test obtained from active plants material on human skin:

Any therapeutic agent before being prescribed for treatment must be tested both in-vitro and in-vivo conditions to prove as a safe agent for the control of disease since detailed in-vitro study on essential oils of Cassia tora, Vitex negundo and Ficus hispida, has indicated their potentiality as their ideal antifungal agent against dermatophytes. These were than subjected for in-vivo investigation so as to confirmed their efficiency as therapeutant for dermatophytic diseases. Most of the earlier investigation was confirmed on in-vitro studies only.

In the present study in-vitro investigations were made on human skin and the first preference the sensitivity of the oil was tested on human skin after 10 days of their application. It was observed that none of the oil produced any allergic response when applied to different persons. Out of 25 persons to whom Cassia tora oil was applied only one complained of mild irritation, one has soothing effect and the rest 23 had no effect. In Vitex negundo

oil ointment out of 25 persons, 22 had no effect, one showed irritation while the rest two had soothing effect.

Ficus hispida oil, out of 25 persons, 2 had mild irritation, 3 had soothing effect and rest 20 had no effect. This shows that these oil could be safely used on human skin without any allergic responses.

Efficiency of the oil for cure of infection :

For in-vivo study mostly experimental animals were employed. Kligman, 1956 induced experimental ringworm infection to mice. Nandi & Bose, 1976 however suggested that these animals have short duration of susceptibility to the disease and therefore they are unsuitable as test animal for in-vivo trials. Others have used guinea pigs for evaluation of topical antimycotic drugs.

In the present investigation observation have been made in the form of percent culture recovery (Wahab et.al., 1982) with the application of the oil. The oil used in the present study was in the form of ointment made in petroleum jelly, which has quick penetration in the skin. Since ointment has animal vegetable, or mineral greases they are more suitable then non-greasy substances. Oil also increased the pliability of dry skin and work as vehicle for penetration of the drugs in skin. As such in the present study the petroleum jelly has been used as base for the preparation of the ointment. In the present study no experimental infection were made on human skin. Only already infected

patients were used for the study, after confirmation for the establishment of infection by particular fungus both through direct examination and culture positive test. Direct examination was made through collection of samples from infected area, their direct examination with 10% KOH solution under the microscope and later by raising their culture on Sabouraud's agar medium. After confirmation In- vivo studies were made by applying the ointment made up with oils and their application twice a day. The results were recorded in terms of percent culture recovery on alternate days from infected area.

During this study infections caused by Trichophyton mentagrophyte, Trichophton rubrum and Microsporum gypseum were treated since during in-vivo test no patient suffering from Microsporum nanum could be found. For in-vivo test Cassia tora oil and Ficus hispida oil were used since these gave better result during in-vitro test as compare to Vitex negundo oil.

In this study complete recovery from Trichophyton mentagrophyte infection was obtained after 21 days treatment with Cassia tora oil ointment and 23 days treatment with Ficus hispida oil ointment. Trichophyton rubrum infection was completely recovered after 23 days treatment of Cassia tora oil ointment and more then 23 days treatment with Ficus hispida oil ointment. Microsporum gypseum was completely cured after 21 days treatment of Ficus hispida and 23 days treatment of Cassia tora oil. This clearly show that Trichophyton could be better cured with Cassia tora oil ointment while Microsporum could be

better cured by Ficus hispida oil ointment. Thus the present investigation suggest that essential oils of Cassia tora pods and Ficus hispida leaves may prove to be promising therapeutant for the cure of dermatomycosis caused by these organisms.

Effect of plant material on the Bio–chemical parameters of blood in Albino rats :

Before effective use of any therapeutant it is necessary to find it's effect when it comes in contact of blood. Since external substances bring changes in the physiology and biochemical distribution within the blood. It also helps in designing the future strategy in drug management. Since rats are used in most of the biological studies and they are easy to be maintained and experimented therefore the effect of the oil on blood were studied on rats. During the past none of the investigator studying on fungitoxic substance has investigated, it's effect on blood, which is very essential while recommending the safe use of a therapeutic agent.

The present study was made on behaviour, food consumption, body weight, total erythrocyte count, total leucocyte count, Hemoglobin content, Blood glucose and Serum cholesterol in Rattus norvgicus. It was observed that the experimental animal behaved normally without any exudation of Saliva from mouth hand legs or body tremors immovability, prostation etc. upto 21 days of treatment. The food consumption of the experimental animals remains normal during the treatment. Although a slightly

higher consumption of food was found incontrolled rat. Since the difference is not much therefore it is quite insignificant.

The body weight of the controlled and treated rat also remains the same, which suggest that difference in food consumption may be temporarily without any significance. When the total erythrocyte count of the controlled rat was compared with that of the treated rat it was found that neither Cassia tora ointment nor Ficus hispida ointment had any ulterior effect on the total erythorocyte count. The total leucocyte count showed a slightly increased in the treated rat as compare to control rat. Which might be due to the injury to rat while applying the ointment. The data obtained on hemoglobin content of the rat showed both the ointment had not adversely affected, the hemoglobin content of the blood in rats. They maintained almost the same level. Similarly there was no effect on the blood glucose or serum cholesterol from either of the ointment. They were maintained almost in the same level as found in the control.

Thus from the over all findings of the present investigations it can be said bot the oils of Cassia tora and Ficus hispida appears to be very positive and promising for the treatment of dermatomycosis caused by Trichophyton mentagrophyte, Trichophyton rubrum and Microsporum gypseum on account of the fact that they have strong fungicidal activity without any irritating or burning effect on the human skin. They have short killing time and have no physiological or biochemical effect in the composition of blood when they come in the direct contact of the blood.

SECTION 6

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